UNIV. OF
TORONTO
LIBRARY

Med
M.

I

The University of Minnesota, Dept. of Anatomy

Toronto Library University

CONTRIBUTIONS
FROM THE
DEPARTMENT OF ANATOMY

VOLUME 6
1918-1919

157477
1/12/20

MINNEAPOLIS
Published by the University of Minnesota
1920

CONTENTS

The following papers have been published during the years 1918-1819. Papers marked with an asterisk (*) are not included in this volume.

1 Adair, Fred L. 1. The ossification centers of the fetal pelvis. *American Journal of Obstetrics and Diseases of Women and Children,* 78:1-25 (1918), 16 figures. pp 1-25

Drips, Della G. 1. *Studies on the ovary of the spermophile with special reference to the corpus luteum. (Abstract.) Proceedings of the American Association of Anatomists. Thirty-fourth session. *Anatomical Record,* 14: 34-35 (1918).

2. Jackson, C. M. 1. How can Anatomy be taught as a science and fulfill its purpose as a foundation for Medicine? *Anatomical Record,* 14:351-358 (1918).

No. 3 2. The postnatal development of the suprarenal gland—and the effects of inanition upon its growth and structure in the albino rat. *American Journal of Anatomy,* 25: 221-289 (1919), 10 figures.

No 4 3. Skill and scholarship as ideals in medical graduate education. *Journal of the American Medical Association,* 73: ~~307-311~~ (1919) pp 1-19

5 Jackson, C. M., and Stewart, C. A. 1. The effects of underfeeding and refeeding upon the growth of the various systems and organs of the body. *Minnesota Medicine,* 1: ~~403-414~~ (1918). pp 1-28

*2. The effects of inanition in the young upon the ultimate size of the body and of the various organs in the albino rat. (Abstract.) Proceedings of The American Association of Anatomists. Thirty-fifth session. *Anatomical Record,* 16: 153 (1919).

No. 6 3. Recovery of normal weight in the various organs of albino rats on refeeding after underfeeding from birth for various periods. *American Journal of Diseases of Children,* 17: ~~329-352~~ (1919). pp 1-28

Johnston, J. B. *1. The history of the nucleus caudatus and the stria terminalis in vertebrates. (Abstract). Proceedings of The American Association of Anatomists. Thirty-fourth session. *Anatomical Record,* 14: 41 (1918).

Lee, Thomas G. *1. The implantation of the blastocyst and the formation of the decidual cavity in Dipodemys. (Abstract.) Proceedings of The American Association of Anatomists. Thirty-fourth session. *Anatomical Record,* 14: 43 (1918).

Myers, J. A. *1. Studies on the mammary gland. IV. The histology of the mammary gland in male and female albino rats from birth to ten weeks of age. (Abstract). Proceedings of The Association of American Anatomists. Thirty-fourth session. *Anatomical Record,* 14: 46 (1918).

No. 7 2. Studies on the mammary gland. IV. The histology of the mammary gland in male and female albino rats from birth to ten weeks of age. *American Journal of Anatomy*, 25: 395-435 (1919), 19 figures, 2 plates. m.

*3. Studies on the mammary gland. V. The effects of inanition on the developing mammary glands in male and female albino rats from birth to ten weeks of age. (Abstract.) Proceedings of the American Association of Anatomists. Thirty-fifth session. *Anatomical Record*, 16: 159 (1919).

No. 8 4. Studies on the mammary gland. V. The effects of inanition on the developing mammary glands in male and female albino rats from birth to ten weeks of age. *American Journal of Diseases of Children*, 17: ~~311-328~~ (1919). pp. 1-20.

No. 9 5. Studies on the mammary gland. VI. The development of the mammary gland from its earliest appearance until the period of pregnancy. *American Journal of Diseases of Children*, 18: ~~4-13~~ (1919) pp. 1-10.

Myers, J. A. and F. J. *1. Studies on the mamary gland. VII. The distribution of the subcutaneous fat and its relation to the developing mammary glands in male and female albino rats from birth to ten weeks of age. (Abstract.) Proceedings of The American Association of Anatomists. Thirty-fifth session. *Anatomical Record*, 16: 159-160 (1919).

No. 10 Norris, E. H. 1. The morphogenesis of the thyroid gland in Squalus acanthias. *Journal of Morphology*, 31: 187-223 (1918), 23 figures.

No. 11 2. The early morphogenesis of the human thyroid gland. *American Journal of Anatomy*, 24: 443-465 (1918), 12 figures.

Rasmussen, A. T. *1. Cyclic changes in the interstitial cells of the ovary in the woodchuck (Marmota monax). (Abstract.) Proceedings of The American Association of Anatomists. Thirty-fourth session. *Anatomical Record*, 14: 48 (1918).

No. 12 2. Cyclic changes in the interstitial cells of the ovary and testis in the woodchuck (Marmota monax). *Endocrinology*, 2: 353-404 411 (1918), 4 plates. m, + paged in

*3 The mitochondrial content of the principal cells of the central nervous system during hibernation in the woodchuck (Marmota monax). (Abstract.) Procedings of The American Association of Anatomists. Thirty-fifth session. *Anatomical Record*, 16; 160-161 (1919).

No. 13 4. The mitochondria in nerve cells during hibernation and inanition in the woodchuck (Marmota monax). *Journal of Comparative Neurology*, 31: 37-49 (1919).

No. 14 Ringoen, Adolph R. 1. The development of the gastric glands in Squalus acanthias. *Journal of Morphology*, 32: 351-377 (1919), 7 figures, 3 plates. m, + paged in.

Tv

Scammon, R. E. *1. On the growth of the various organs of the body during fetal life. (Abstract.) Proceedings of The American Association of Anatomists. Thirty-fourth session. *Anatomical Record,* 14:49 (1918).

*2. On the development and finer structure of the sucking-pad (Corpus adiposum buccae). (Abstract.) Proceedings of the American Association of Anatomists. Thirty-fourth session. *Anatomical Record,* 14:49 (1918).

No. 15 3. On the development and finer structure of the corpus adiposum buccae. *Anatomical Record,* 15:267-287 (1919), 9 figures.

*4. Some peculiarities of the growth of the human uterus. (Abstract.) Proceedings of The American Association of Anatomists. Thirty-fifth session. *Anatomical Record,* 16:163 (1919).

No. 16 5. Some graphs and tables illustrating the growth of the human stomach. *American Journal of Diseases of Children,* 17:395-422 (1919). p. 1-28

*6. A simple mounting for demonstration slides. *Anatomical Record,* 17:105-106 (1919).

Scammon, R. E., and Norris, E. H. *1. On the time of obliteration of the fetal blood-passages after birth. (Abstract.) Proceedings of The Association of American Anatomists. Thirty-fourth session. *Anatomical Record,* 14:49 (1918).

No. 17 2. On the time of the post-natal obliteration of the fetal blood-passages (foramen ovale, ductus arteriosus, ductus venosus). *Anatomical Record,* 15:165-180 (1919), 3 figures.

Stewart, C. A. *1. Weights of the various parts of the brain in normal and underfed albino rats at different ages. (Abstract.) Proceedings of The American Association of Anatomists. Thirty-fourth session. *Anatomical Record,* 14:51 (1918).

No. 18 2. Changes in the relative weights of the various parts, systems and organs of young albino rats underfed for various periods. *Journal of Experimental Zoölogy,* 25:301-353 (1918). 1 figure.

No. 19 3. Weights of various parts of the brain in normal and underfed albino rats at different ages. *Journal of Comparative Neurology,* 29:511-528 (1918).

No. 20 4. Changes in the weights of the various parts, systems and organs in albino rats kept at birth weight by underfeeding for various periods. *American Journal of Physiology,* 48:67-78 (1919).

No. 1 1

[*Reprinted from the American Journal of Obstetrics and Diseases of Women and Children,*
Vol. LXXVIII, No. 2, 1918.]

THE OSSIFICATION CENTERS OF THE FETAL PELVIS.*

BY

FRED L. ADAIR, M. D.,

Associate Professor of Gynecology and Obstetrics, University of Minnesota Medical School,
Minneapolis, Minnesota.

(With sixteen illustrations.)

A NUMBER of studies have been made of the centers of ossification in the pelvis. So far no one has made use of combined methods in working out a series and no one has investigated so large a series covering this period of fetal life which extends from the 56th to the 198th day. It seemed desirable to settle, if possible, the time and order of the appearance of these different centers. The material for this study is derived from personal sources and also from the laboratory of the Department of Obstetrics and Gynecology and chiefly from the Institute of Anatomy of the University of Minnesota. I am deeply indebted to Dr. R. E. Scammon, whose patience and persistent help has enabled me to complete this present study.

THE OSSIFICATION CENTERS OF THE PELVIS.

The bony pelvis should be regarded as developing from two sources: First, the lower portion of the vertebral column (sacrum and coccyx); second, the upper portion of the lower extremities (pelvic girdle). The development of the sacrum and coccyx is analogous to that of the other vertebræ.

Early in the embryonic life the chorda dorsalis develops in the anterior portion of the embryo, in close association with the disappearing neurenteric canal. It extends caudad and seems to be derived from the primitive streak. The cylindrical chorda with

---

* Read at the Forty-third Annual Meeting of the American Gynecological Society, May 16–18, 1918.

Copyright, William Wood & Company.

its polygonal cells and encircling membrane reaches the height of its development about the fourth week. During this period there are some condensations of the axial mesenchyme marking off the spinal column. The vertebral bodies and the intervertebral discs form around the notochord. It disappears in the former about the time ossification begins though persisting in the intervertebral discs as the nucleus pulposus.

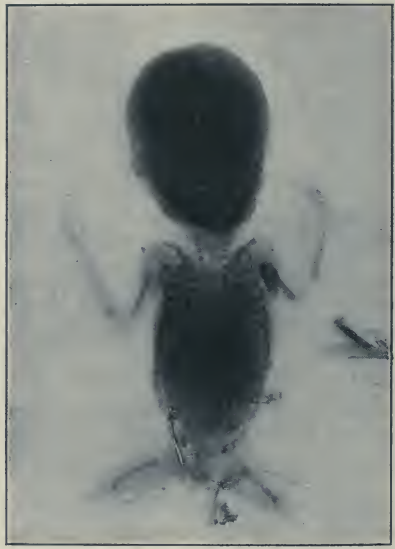


FIG. 1 (12).—Age 82 days. C.R. length 65 mm. Sex ? The iliac first and second median and first lateral sacral centers are present.

These segments of the axial mesenchyme are called scleromeres and from these the blastemal vertebræ are formed. On each side of these primitive vertebræ three centers of chondrification appear, one for the body, a second for the neural process and a third for the costal process. The two body centers soon unite. The sacral and coccygeal vertebræ show variations depending chiefly on the development of the neural and costal processes.

The neural cartilages of the sacrum produce the radicular, articular, transverse and laminary processes. The costal processes fuse with the transverse processes of the neural cartilages. Fusion,

NOTE.—In all the plates the numbers in parentheses refer to the numbers of the specimens as given in the tables.

The figures are arranged according to the ages of the fetus, Fig. 1 being the youngest and Fig. 16 the oldest.

of the ends of the costal cartilages, produces that part of the sacrum which articulates with the ilium.

The neural processes appear only in the first two coccygeal vertebræ and develop incompletely. They are represented by the cornua of the adult coccyx.

Costal processes appear only in the blastemal stage except in the first coccygeal where they form, together with transverse processes, the adult transverse processes.

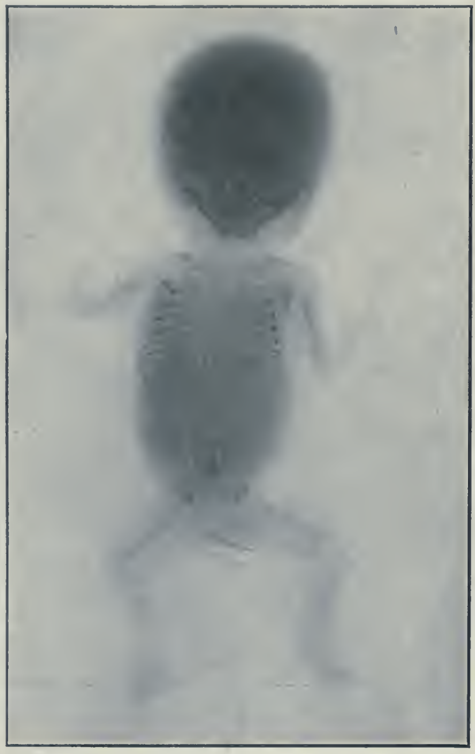


FIG. 2 (8).—Age 87 days. C.R. length 75 mm. Sex F. The iliac and the first and second median sacral centers are present.

All of the centers of the sacrum fuse to form a single cartilaginous sacrum.

"Centers of ossification correspond in general with centers of chondrification but as in the case of the vertebral bodies and the more distal sacral neurocostal processes, a single center of ossification may represent two centers of chondrification(1)."

The remaining portion of the pelvis passes through the blastemal stage at the completion of which three cartilaginous centers are found. These are barely visible in an embryo of 11 mm. Each of these centers represents one of the three pelvic bones, ilium, ischium, or pubis.

They gradually assume the form seen in the adult so that a miniature cartilaginous pelvis is formed closely resembling the adult type.

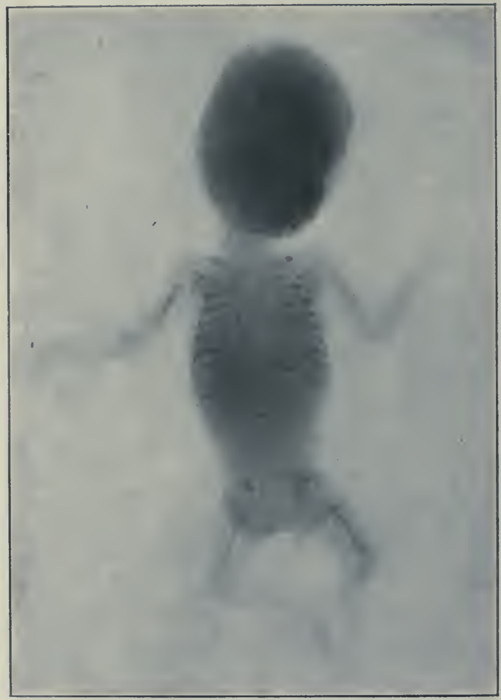


FIG. 3 (13).—Age 88 days. C.R. length 76 mm. Sex F. The iliac and the first three median sacral centers are present.

A shallow acetabulum is developed in embryos 15–20 mm. long by the outgrowth of a process from each of the three cartilages, which gradually deepens during the third month.

The union of the ilium and the ischium takes place before either unites with the pubis. The pubic symphysis is formed about the end of the second or beginning of the third month. Hyaline and fibrocartilage are successively differentiated. Separate ossification centers are formed for each one of the three bones. According to

Bardeen(1) the center appears in the ilium about the 56th day, that in the ischium on the 105th day and in the pubis in the fourth or fifth month.

The same author states that each body of the five sacral vertebræ has a primary center of ossification. There is also one for each half of the neural arch of all five. There is greater variability in the costal processes. Their centers usually appear in three or four but may be in only two or in all five sacral vertebræ.

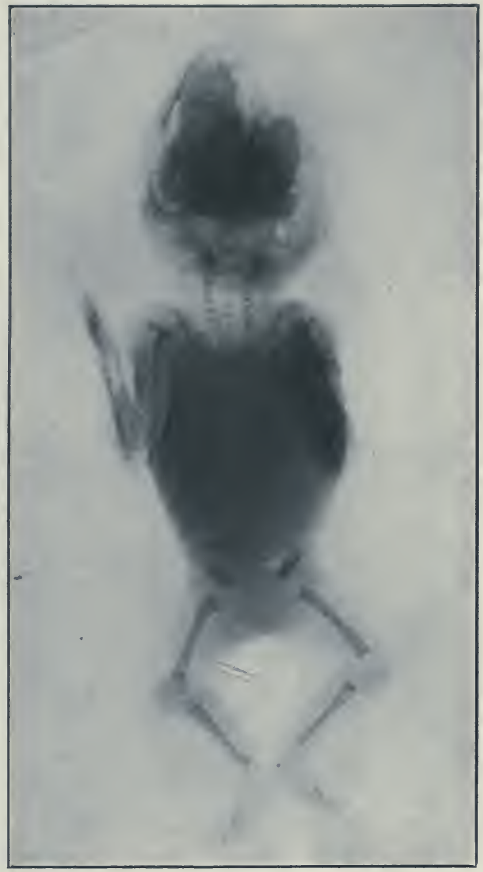


FIG. 4 (10).—Age 89 days. C.R. length 77 mm. Sex F. The iliac, first four median sacral and one pair of lateral sacral centers are present.

The time of appearance of the sacral centers is in the fourth month for the vertebral bodies and in the fifth or sixth month in their arches. The first, second and third costal centers appear in the fifth, sixth and seventh months respectively. All other pelvic centers appear after birth as a rule.

Rambaud and Renault(2) in 1864 published the results of their study by dissection of the ossification centers in the human skeleton. They state that the iliac center is present on the 45th day and that its longest diameter is 4 to 5 mm. at the end of the second month. A second center appears in ischium usually in the third but rarely in the fourth month and is located between the tuberosity and ischial spine. A third center appears in the pubes during the fourth month sometimes during the fifth month at the point of junction between the rami opposite the upper angle of the obturator foramen.

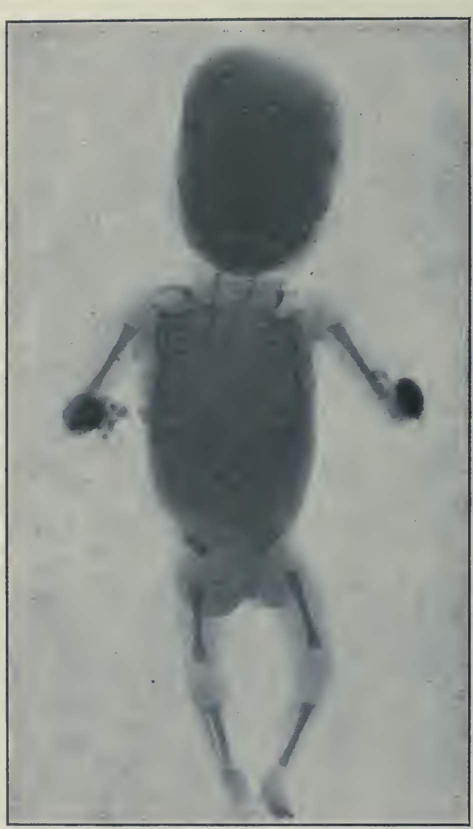


FIG. 5 (11).—Age 90 days. C.R. length 79 mm. Sex F. The iliac, first three median sacral and first lateral sacral centers are present.

In the fourth month the centers for the bodies of the first three sacral vertebræ appear. In the fifth month the centers appear for the neural and costal processes of the first three sacral vertebræ. During the fifth or sixth months the centers of the costal processes for the first three sacral vertebræ appear as also do the centers for the body of the fourth as well as that of the neural arch. No new centers appear until after birth.

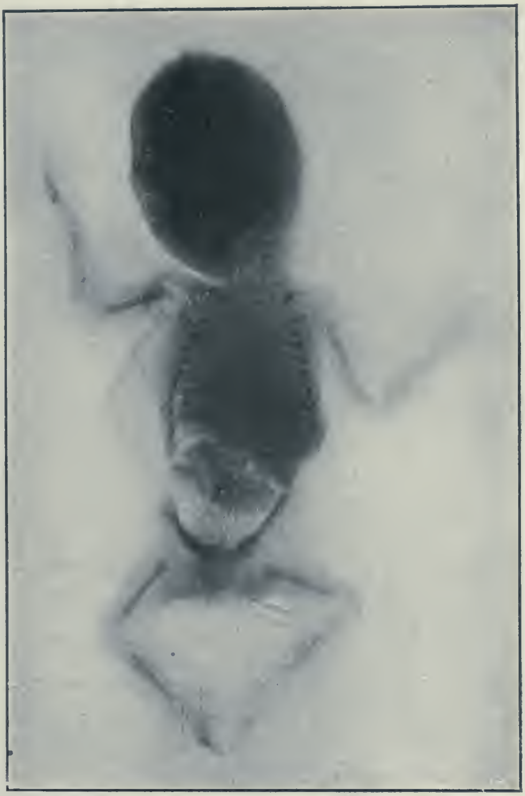


FIG. 6 (1).—Age 90 days. C.R. length 79 mm. Sex M. The iliac, first three median sacral and one pair of lateral sacral centers are present.

Peterson(3) published in 1893 the results of his work with fifteen embryos from four to eight weeks' gestation having a N.R. length of 9.1 mm. to 25 mm., based on microscopic examination and one paper reconstruction.

The first trace of a pelvic "*anlage*" appears about the fifth week in a fetus having a N.R. length of 12.6 mm. From the acetabulum three processes diverge each with a precartilaginous nucleus. One of these represents the ilium, shows the greatest development and runs almost parallel to the first sacral vertebra. This sends out processes toward each of the other two pelvic centers, the most marked going toward the ischium.

The cartilaginous pelvis is completed in a 29-mm. embryo early in the third month. Thus the cartilaginous pelvis is developed in a period of about three and one-half weeks. Peterson regards the pelvis as peripheral in origin because the first "*anlage*" is at the acetabulum and has no connection with the vertebral bodies. He considers that the direction of growth from the anlage is determined by the three large nerves, N. cruralis, obturatorius and ischiadicus.

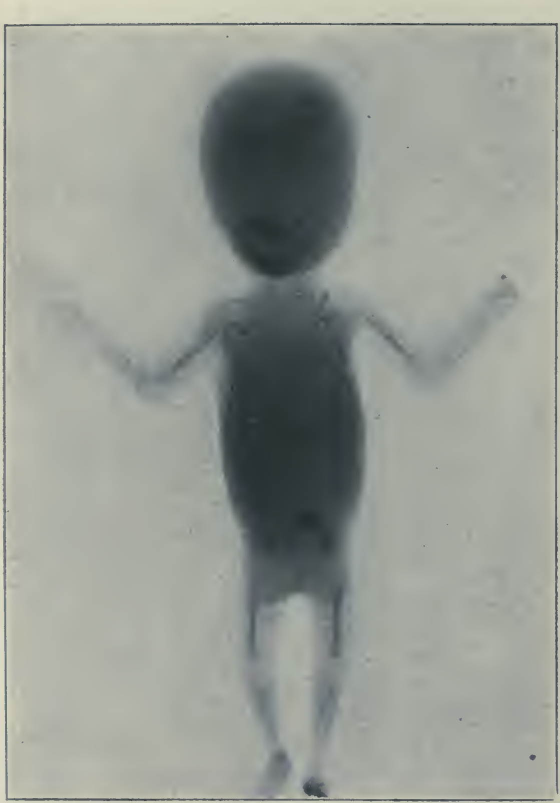


FIG. 7 (7).—Age 92 days. C.R. length 84 mm. Sex F. The iliac centers and the first two median sacral centers are present.

Bade(6) in 1899 reported his investigations bassd on a röntgenographic study of the human skeleton.

He studied homologous twins of about 12 weeks' gestation which showed ossification centers for the bodies of the first three sacral vertebræ. The ossification center for the ischium begins to be formed about the 15th week. The first sacral centers appear in the vertebral bodies after all the thoracic and lumbar vertebræ have three centers. The sacral centers develop slowly and show hardly any growth for 3 or 4 weeks after their appearance. There is little growth up to 18 or 20 weeks and then they develop rapidly at which time the bodies of the first four sacral vertebræ each have their centers. That of the fifth sacral does not appear until about 30 weeks. Bade found an iliac center in a fetus of 11 weeks' gesta-

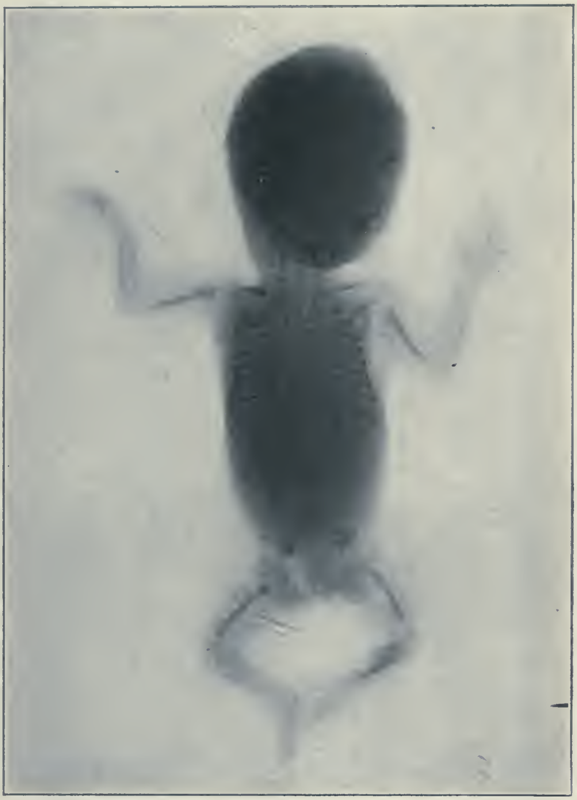


FIG. 8 (5).—Age 92 days. C.R. length 85 mm. Sex F. The iliac center, three median sacral and one pair of lateral centers are present.

tion having a length of 7.1 cm. and weighing 25 grams. The ischial center in an embryo of 15–16 weeks' gestation appears in the descending ramus and the pubic center in its horizontal ramus about the 25th week

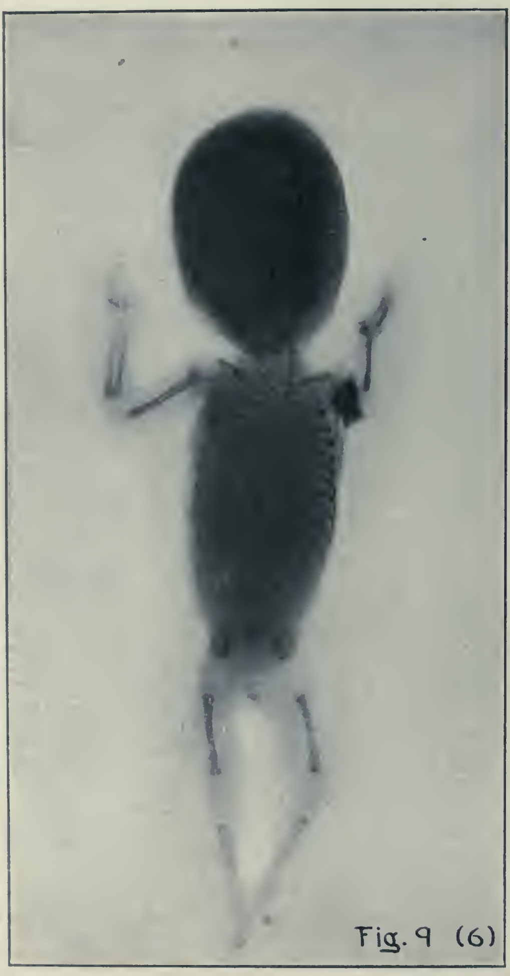


FIG. 9 (6).—Age 96 days. C.R. length 96 mm. Sex M. The iliac center, four median and two pair of lateral sacral centers are present.

Hagen(5), who based his work on microscopic study and reconstruction, states that the pelvis in its cartilaginous development comes from two lateral "*analgen*" which are not connected with each other or with the sacrum. In these, three parts may be seen, which later develop into the ilium, ischium and pubis. There are no lines of separation but on the sagittal surface three segments are apparent. He thinks the pelvis in its growth first approaches the second sacral vertebra and extends upward to the first. He is of the opinion that there is little change in the plane of the inlet because it is already in the final relation to the sacrum.

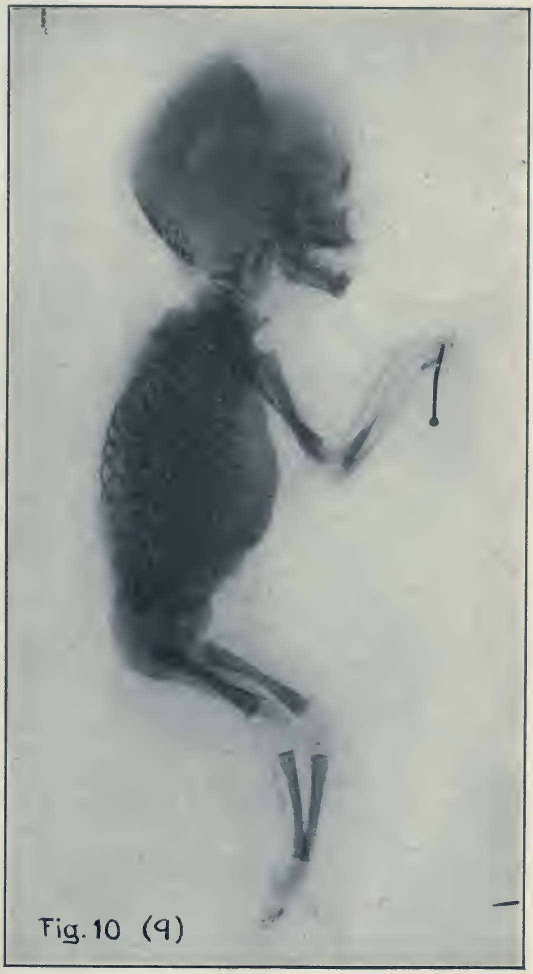


FIG. 10 (9).—Age 98 days. C.R. length 100 mm. Sex F. Lateral view of fetus showing fan shape of iliac center and the much smaller round ischial center.

Lambertz(6) also studied the fetal skeleton by means of the *x*-ray. He thinks that all of the vertebræ which show ossification during fetal life have centers at the end of the third month except possibly the fourth and fifth sacral and the first coccygeal.

The cartilaginous "*anlagen*" of the sacrum consist of five vertebræ connected by intervertebral discs. The lateral masses contain rudimentary ribs which are seen in the first two sacral vertebræ. The first sacral, like the last lumbar, has three centers which are macroscopically visible in the third month.

The sacral centers appear during the following periods of development. At the end of the third month they are present in the bodies of the first three sacral vertebræ and in the arches of the first; in the fourth month, centers show in the arches of the second; during the fifth month they appear in the arches of the third and the body of the fourth, and at the beginning of the sixth month they exist in the arches of the fourth sacral vertebra.

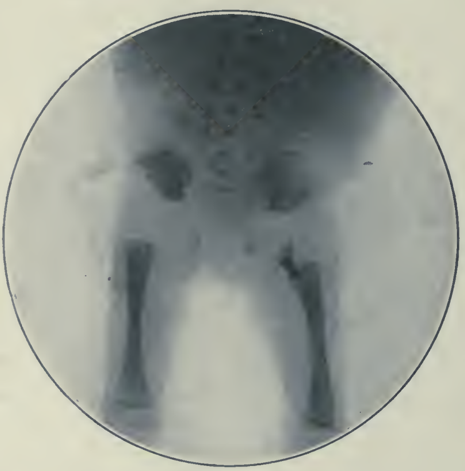


FIG. 11 (9).—View of pelvis showing the iliac, the median centers of four and lateral centers of three sacral vertebræ and the pair of ischial centers.

The os coxæ is represented at first by three separate cartilaginous parts which are united to the vertebral column at the end of the third month.

Ossification begins in the ilium, about the ninth week, near the acetabulum in the region of the incisura ischiadica major. Lambertz thinks that a second iliac center is present dorsal to the first which is quiescent until the middle of the third month when it grows and unites with the first. A third center, located ventrally appears about the end of the third or beginning of the fourth month, and lies ventral to those mentioned above.

These two centers correspond respectively to the spina iliaca posterior inferior and anterior superior.

The center, in the descending ischial ramus, appears at the end of the fourth or beginning of the fifth month and the pubic center is present in the horizontal ramus between the end of the fifth and the end of the seventh month.

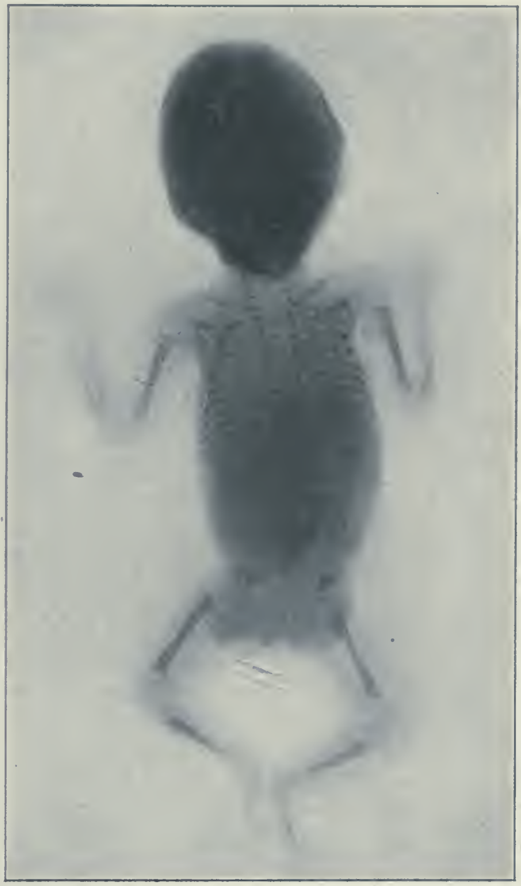


FIG. 12 (14).—Age 98 days. C.R. length 100 mm. Sex M. The iliac and first two median sacral centers are the only centers present.

Mall(7) based his work on a study of sixty embryos with a C.R. length of from 10 to 110 mm., and used embryos made transparent by the Schultze method. He states that the ossification center of the ilium appears about the 56th day in an embryo with a C.R. length of 31 mm. The ischial center appears about the 105th day.

The arches of the first three sacral vertebræ have centers on the 72d day in an embryo with a C.R. length of 54 mm. The body of the first sacral vertebra shows an ossification center about the 58th day in an embryo with a C.R. length of 34 mm.

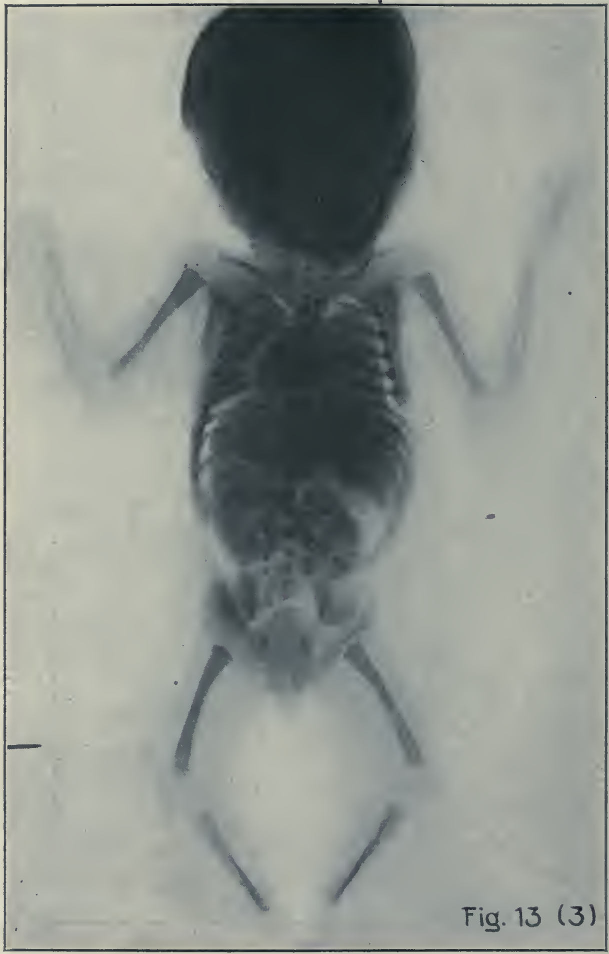


FIG. 13 (3).—Age 105 days. C.R. length 110 mm. Sex M. The iliac, first four median and three pair of lateral sacral centers and both ischial centers are present.

Hess(8) has recently published an article based on a study, of the fetal ossification centers, by means of the *x*-ray.

So far as the centers of the pelvis are concerned his statements are not very clear or exact. He states that the time of appearance of the ossification centers in the axial skeleton are as follows:

Bodies from lower cervical to upper sacral	11th week.
Bodies from upper cervical to lower sacral	12th week.
Body of fifth sacral	13th to 28th week.
Body of first coccygeal	37th to 40th week.

> The iliac center appears in the ninth week.
> The ischial center appears in the descending ramus 16th to 17th week.
> The pubic center is located in the horizontal ramus and appears during the 21st to 28th week.

Obata(9), in a study of R. Meyer's material which comprised both transparent embryos and serial sections, concludes that the iliac center appears macroscopically in embryos having a C.R. length of 35 mm. and microscopically in embryos with a C.H. length of 60 mm.

The first median sacral appears in embryos with a C.R. length of 50 mm., the fifth is evident in embryos with C.R. length of 155 to 190 mm.

The first lateral center is present in those with a C.R. length of 65 to 90 mm. and those of the fifth sacral appear in embryos with a C.R. length of from 190 to 230 mm.

The ischial centers appear in embryos having a C.R. length of 120 mm.

The pubic centers are apparent in the embryos having a C.R. length of 160 mm. or more.

The first coccygeal center was present at times in the new-born. All other centers were generally present at birth.

All centers except those of the lateral sacral masses might be present in embryos having a C.R. length of 190 mm. and were generally present in those having a length greater than 230 mm.

In so far as comparison of Obata's series with mine is possible, the results are very much alike.

I have made use of three methods in studying the ossification centers of the pelvis. 1. Transparent embryos. 2. X-ray. 3. Serial sections.

There is a series of 35 transparent specimens prepared by the Schultze method and preserved in glycerine. There are no data about these specimens and in some of them the sex could not be determined because they were too small and already made transparent when this study was undertaken. The following Table (No. I) gives the results of this observation.

TABLE I.—CENTERS OF OSSIFICATION SHOWN BY TRANSPARENT SPECIMEN.

Number	C.R., mm.	Age, days	Sex	Iliac	Sacral		Ischial	Pubic
					Median	Lateral		
A[1]	25	56	?	0	0	0	0	0
Z[2]	30	60	?	+	0	0	0	0
B[1]	32	63	M.	0	0	0	0	0
C[1]	35	65	?	0	0	0	0	0
D[1]	38	67	M.	+	0	0	0	0
F[1]	40	68	M.	+	0	0	0	0
G[1]	44	70	M.	+	0	0	0	0
A	48	72	?	+	0	0	0	0
L	48	72	M.	+	0	0	0	0
N	51	74	?	+	1	0	0	0
Q	52	76	?	+	1	0	0	0
F	55	78	?	+	1	0	0	0
K[1]	58	79	M.	+	0	0	0	0
B	65	82	?	+	3	2	0	0
Z[1]	68	84	?	+	2	1	0	0
P	70	85	F.	+	3	1	0	0
S	73	86	?	+	?	?	0	0
G	78	89	M.	+	3	1	0	0
D	80	90	F.	+	4	2	0	0
C	81	91	F.	+	3	2	0	0
O	82	91	?	+	3	1	0	0
M	84	92	M.	+	3	2	0	0
E[1]	88	93	M.	+	?	?	+	0
X	90	94	F.	+	3	1	+	0
U	92	95	M.	+	4	2	+	0
E	100	98	F.	+	4	3	+−6	0
W	102	99	M.	+	4	3	+	0
T	105	100	M.	+	4	3	+	0
V	109	104	M.	+	4	3	+	0
K	110	105	F.	+	4	2	+	0
Y	120	112	F.	+	4	1	+	0
R	123	113	M.	+	4	3	+	0
L[1]	133	119	M.	+	5	4	+	0
J	150	129	F.	+	4	4	+	+
I	181	148	F.	+	4	4	+	+

N.B.—The age was estimated from the C.R. length from Mall's tables.

[1] Anomaly. All median vertebral centers hypoplastic.

From this series it is apparent that the iliac center is present on the 60th day. The first median sacral is present by the 74th day and that of the second and third vertebræ appear shortly after this. The lateral centers appear about the same time in the first two sacral vertebræ soon after the appearance of their median centers.

There seems to be some variation in the time of appearance of these centers especially those of the sacrum and in particular the lateral sacral centers. The ischial center is evident about the 94th day but the pubic center does not appear until about the 129th day.

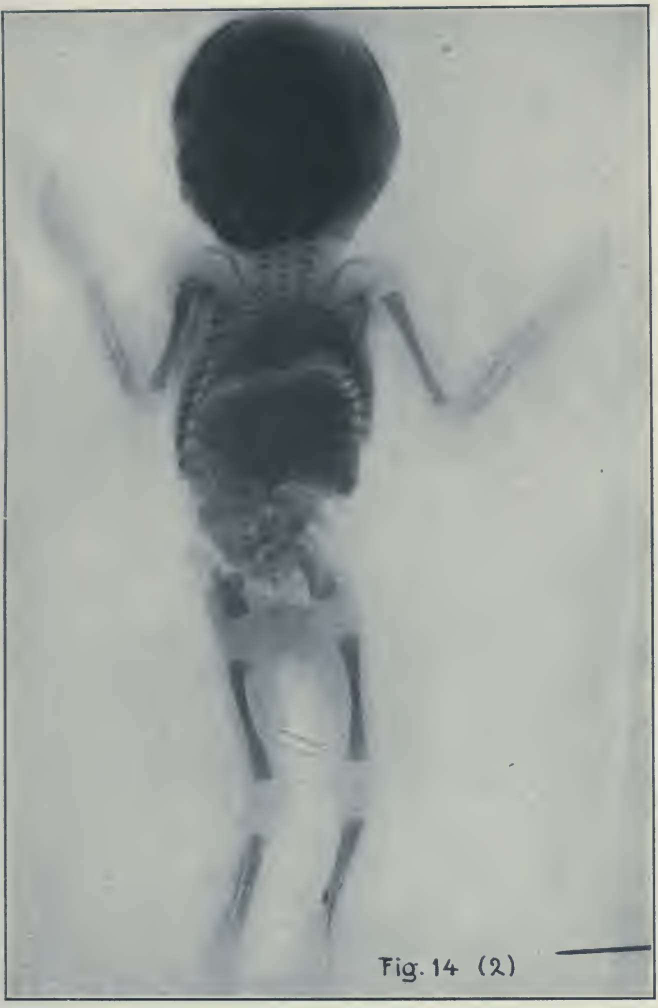


FIG. 14 (2).—Age 108 days. C.R. length 115 mm. Sex F. The iliac, the median centers of the first four sacral vertebræ are present. The ischial centers are just appearing.

In the oldest specimen of the series (148 days), the iliac, four median sacral, presumably four lateral sacral, the ischial and pubic centers are all present. All pelvic centers which appear before birth except those of the last sacral and the first coccygeal are present by the end of the 21st week of fetal life.

The specimens studied by means of the *x*-ray, which was the second method used, numbered twenty-four—four of these had to be discarded because of the technical difficulties in securing good pictures. Table II gives the results of the study of the twenty available plates.

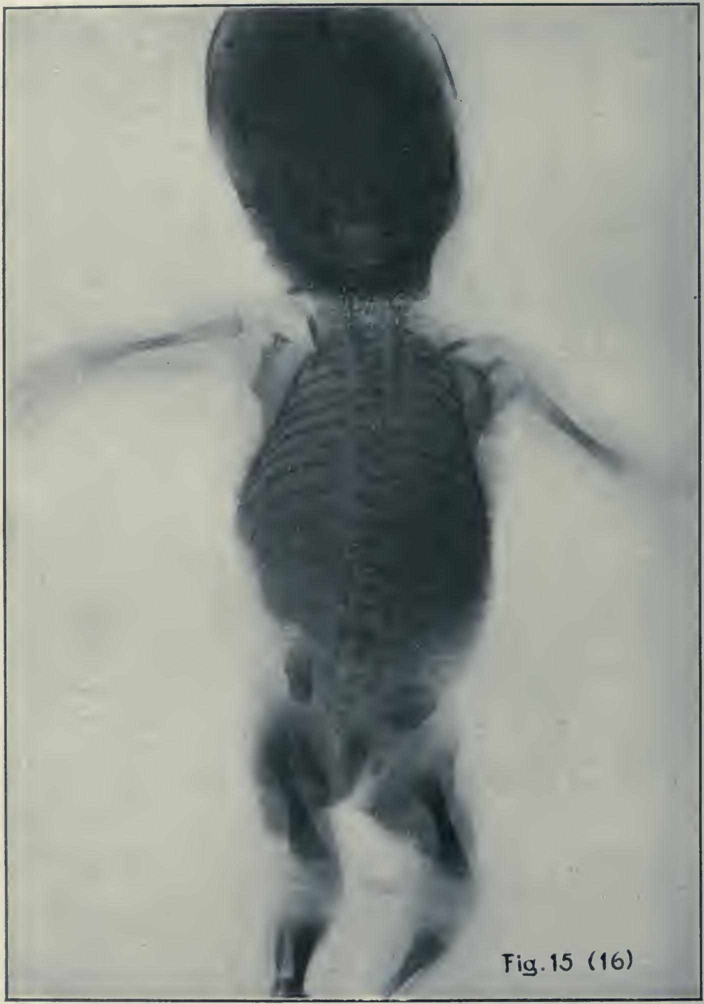


FIG. 15 (16).—Age 117 days. C.R. length 130 mm. Sex M. The iliac, the median and lateral centers of the first four sacral and both ischial centers are evident.

TABLE II.—CENTERS OF OSSIFICATION SHOWN BY X-RAY.

Number	Length, mm.						Sacral			
	C.H.	C.R.	O.R.	Age	Sex	Iliac	Median	Lateral	Ischial	Pubic
12	100	65	53	82	?	+	2	1	0	0
8	110	75	63	87	F.	+	2	0	0	0
13	100	76	60	88	F.	+	3	0	0	0
10	123	77	64	89	F.	+	4	1	0	0
11	123	79	68	90	F.	+	3	1	0	0
1	110	79	70	90	M.	+	3	1	0	0
7	117	84	64	92	F.	+	2	0	0	0
5	116	85	67	93	F.	+	3	1	0	0
6	140	96	72	97	M.	+	4	2	0	0
9	157	100	79	98	F.	+	4	3	+	0
14	141	100	80	98	M.	+	2	0	0	0
3	170	110	94	105	M.	+	4	3	+	0
2	165	115	83	108	F.	+	4	0	+	0
19	. . .	120	. . .	112	F.	+	3	2	+	0
20	. . .	140	. . .	124	F.	+	3	3	+	0
17	. . .	165	. . .	138	M.	+	3	3	+	0
18	. . .	180	. . .	147	M.	+	4	3	+	0
22	. . .	200	. . .	160	M.	+	4	3	+	+
21	. . .	240	. . .	190	M.	+	5	4	+	+
16	200	130	109	117	M.	+	4	4	+	0

The *x*-ray gives data which correspond quite closely with that for the transparent specimens.

The youngest specimen, about 82 days old, shows the presence of the iliac as well as two median and one pair of lateral sacral centers. The ischial center appears about the 98th day, when four median and three lateral sacral centers are present. None of these show a pubic center. The sacral centers show considerable variation as in the previous table.

The third method of investigation was by means of microscopic serial sections of fetal pelves. Twenty-five specimens, including the one from which a wax model was made, were studied in this wya.

The results of this study are given in Table III.

In the following tables the abbreviation P.A. indicates that the primary areola is present but that no ossification has taken place. The letters L.C.C. mean that enlarged cartilage cells were seen in groups at the site of the future center of ossification. From this series it is evident that no ossification center is present in the ilium until the 65th day at which time no other pelvic centers are present.

The next centers appear about the 80th day in the bodies of the first two sacral vertebræ. The first two pairs of lateral sacral centers appear about the 128th day. The ischial centers are present about the 96th day and the pubic centers are evident about the 141st day. There are present on the 168th day the iliac, ischial and pubic centers as well as four median and lateral sacral centers.

As in the two preceding series we find that the pubic, ischial and iliac centers show little variation as to time of appearance. The sacral median and especially the lateral centers show a great deal of variation in the time but not in the order of their appearance. We have not seen a single instance where a lower center appeared before those of the vertebræ lying above.

TABLE III.—OSSIFICATION CENTERS BY SERIAL SECTIONS.

Number	C.R. mm.	Age	Sex	Iliac	Sacral		Ischial	Pubic
					Median	Lateral		
H259	30	61	F.	P.A.	0	0	0	0
H98	30	61	M.	P.A.	0	0	0	0
H108	30	61	F.	L.C.C.	0	0	0	0
H57	31	62	F.	P.A.	0	0	0	0
H16	33	63	F.	P.A.	0	0	0	0
H298	35	65	M.	P.A.	0	0	0	0
H313	35	65	M.	P.A.	0	0	0	0
H51	35	65	M.	+	0	0	0	0
H122	39	68	M.	+	0	0	0	0
H8	41	69	M.	+	0	0	0	0
H12	41	69	F.	+	0	0	0	0
H100	43	70	F.	+	0	0	0	0
H121	46	72	M.	+	0	0	0	0
H115	50	74	M.	+	0	0	0	0
H11	60	80	M.	+	2	0	0	0
H55	65	82	M.	+	2	0	0	0
H26	65	82	M.	+	2	0	0	0
H31	93	96	F.	+	2 P.A.	1 P.A.	+	0
H34	120	111	F.	+	?	?	+	0
H49	128	117	F.	+	2 1 P.A.	1 1 P.A.	+	0
H54	150	129	F	+	3	2	+	0
H35	170	141	F	+	4	3	+	+
H37	172	142	F.	+	4	4	+	+
H275	183	149	M.	+	4	3	+	+
H36	210	168	F.	+	4	4	+	+

In the following Table No. IV, the three series are combined in an attempt to group all the specimens studied according to age and sex. This shows again considerable variation in the time when the sacral centers, especially the lateral ones, appear. No constant sex variation is apparent so far as can be judged from this series.

In this table all those specimens which are designated by any letter other than H belong to the series of transparent embryos, those having Arabic numerals are in the *x*-ray series and all specimens indicated by H with an appended numeral are in the serial section series.

This table shows that the iliac center in all probability appears between the 60th and 65th day of fetal life and is constantly present thereafter.

The next center to appear is that of the body of the first sacral vertebra which occurs about the 74th day after which time it is always present.

There is quite a bit of variation in the number of median sacral centers present at different ages.

The earliest date of the appearance of the lateral sacral centers was the 82nd day. These centers show a great deal of variation not only in the time of their appearance but also in the number present at the different ages.

The ischial center appears about the 94th to 98th day and is constantly present after that time.

TABLE IV.—COMBINED SERIES.

Number	C.R. mm.	Age, days	Sex	Iliac	Sacral		Ischial	Pubic
					Median	Lateral		
A^1	25	56	?	0	0	0	0	0
Z^2	30	60	?	+	0	0	0	0
H259	30	61	F.	P.A.	0	0	0	0
H98	30	61	M.	P.A.	0	0	0	0
H108	30	61	F.	L.C.C.	0	0	0	0
H57	31	62	F.	P.A.	0	0	0	0
B^1	32	63	M.	0	0	0	0	0
H16	35	63	F.	P.A.	0	0	0	0
H298	35	65	M.	P.A.	0	0	0	0
C^1	35	65	?	0	0	0	0	0
H313	35	65	M.	P.A.	0	0	0	0
H51	35	65	M.	+	0	0	0	0

TABLE IV.—COMBINED SERIES.—*Continued.*

Number	C.R. mm.	Age, days	Sex	Iliac	Sacral		Ischial	Pubic
					Median	Lateral		
D^{1}	38	67	M.	+	0	0	0	0
H122	39	68	M.	+	0	0	0	0
F^{1}	40	68	M.	+	0	0	0	0
H8	41	69	M.	+	0	0	0	0
H12	41	69	F.	+	0	0	0	0
H100	43	70	F.	+	0	0	0	0
G^{1}	44	70	M.	+	0	0	0	0
H121	46	72	M.	+	0	0	0	0
A	48	73	?	+	0	0	0	0
L	48	73	M.	+	0	0	0	0
M	50	74	M.	+	0	0	0	0
N	51	74	?	+	1	0	0	0
Q	52	76	?	+	1	0	0	0
F	55	78	?	+	1	0	0	0
K^{1}	58	79	M.	+	0	0	0	0
H11	60	80	M.	+	2	0	0	0
H55	65	82	M.	+	2	0	0	0
H26	65	82	M.	+	2	0	0	0
12	65	82	?	+	2	1	0	0
B	65	82	?	+	3	2	0	0
Z^{1}	68	84	?	+	3	1	0	0
P	70	85	F.	+	3	1	0	0
S	73	86	?	+	?	?	0	0
8	75	87	F.	+	2	0	0	0
13	76	88	F.	+	3	0	0	0
10	77	89	F.	+	4	1	0	0
G	78	89	M.	+	3	1	0	0
11	79	90	F.	+	3	1	0	0
1	79	90	M.	+	3	1	0	0
D	80	90	F.	+	4	2	0	0
C	81	91	F.	+	3	2	0	0
O	82	91	?	+	3	1	0	0
M	84	92	M.	+	3	2	0	0
7	84	92	F.	+	2	0	0	0
5	85	93	F.	+	3	1	0	0
E^{1}	88	94	M.	+	?	?	+	0
X	90	95	F.	+	3	1	+	0
U	92	96	M.	+	4	2	+	0
H31	93	96	F.	+	2 P.A.	1 P.A.	+	0
6	96	97	M.	+	4	2	0	0
9	100	98	F.	+	4	3	+	0
14	100	98	M.	+	2	0	0	0

TABLE IV.—COMBINED SERIES.—*Continued.*

Number	C.R., mm.	Age, days	Sex	Iliac	Sacral		Ischial	Pubic
					Median	Lateral		
E	100	98	F.	+	4	3	+	0
W	102	99	M.	+	4	3	+	0
T	105	100	M.	+	4	3	+	0
V	109	103	M.	+	4	3	+	0
K	110	105	F.	+	4	2	+	0
3	110	105	M.	+	4	3	+	0
2	115	108	F.	+	4	0	+	0
Y	120	111	F.	+	4	1	+	0
H34	120	111	F.	+	?	?	+	0
19	120	112	F.	+	3	2	+	0
R	123	113	M.	+	4	3	+	0
H49	128	117	F.	+	2 1 P.A.	1 1 P.A.	+	0
16	130	117	M.	+	4	4	+	0
L^{1}	133	119	M.	+	5	4	+	+
20	140	124	F.	+	3	3	+	0
J	150	129	F.	+	4	4	+	+
H54	150	129	F.	+	3	2	+	0
17	165	138	M.	+	3	3	+	0
H35	170	141	F	+	4	3	+	+
H37	172	142	F.	+	4	4	+	+
18	180	147	M.	+	4	3	+	0
I	181	148	F.	+	4	4?	+	+
H275	183	149	M.	+	4	3	+	+
22	200	160	M.	+	4	3	+	+
H36	210	168	F.	+	4	4	+	+
21	240	190	M.	+	5	4	+	+

The pubic center is constantly evident after the 119th day at which time the ischial, iliac as well as four median and lateral sacral centers may be present. The existence of all these centers may be deferred until the 142d or even the 168th day of fetal life.

This series of eighty fetuses studied by three different methods, all of which give practically the same results, should make the above statements quite conclusive.

It is clear that practically all of the pelvic ossification centers are present at the end of the 20th week of gestation and that the appearance of the first center occurs about the 9th week, in the iliac cartilages.

The order of the appearance of these centers is: the iliac, the first two, three or even four median sacral, one or two lateral sacral, the ischial, more of the sacral centers and finally the pubic center.

The median and lateral sacral centers always proceed, in this series, in their appearance from above downward. In no specimen did the lateral sacral center appear before one was present in the body of the corresponding vertebra.

I was unable to confirm Lambertz's observation of secondary iliac centers of ossification.

There were none in any of the specimens studied by any of the three methods. Our *x*-ray plates might not have shown them because only frontal views were taken.

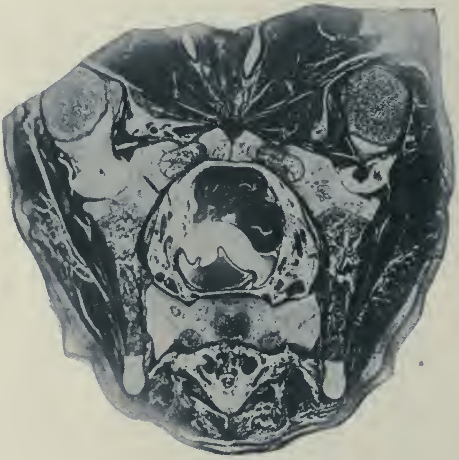


Fig. 16 (H 36).—Age 168 days. C.R. length 210 mm. Sex F. Photograph of microscopic section through the pelvis. The ossification centers of the femora are distinct. The iliac, median and lateral sacral and both pubic centers are easily seen.

Our series of serial sections was studied to determine this point and in none of them were we able to find isolated secondary iliac centers.

Early in the second month the center sometimes has a Y-shape evidently due to outgrowths, in two directions, from the primary center. The opening of the Y looks upward and forward and the ends of the outgrowths are rather club shaped. It is possible that these ends might show in the *x*-ray plate as isolated centers of ossification but microscopically they are connected with the primary center. One specimen in the series of transparent embryos showed a similar formation—two large centers united with a narrow strand of similar structure.

In the latter half of the second month the center becomes more fan shaped in appearance which form persists for some time as seen in the accompanying plates.

CONCLUSIONS.

I. The first ossification center of the pelvis to appear is in the ilium about the 60th to the 65th day of fetal life in embryos with a C.R. length of from 30 mm. to 35 mm. There are no separate secondary centers.

II. The median center of the first sacral vertebra is the next to appear about the 74th to 76th day in embryos having a C.R. length of 51 to 52 mm.

III. The lateral sacral centers first appear when two or three median centers are present, in embryos eighty to eighty-two days old having a C.R. length of 65 mm.

IV. The ischial center appears about the 94th to 98th day in embryos whose C.R. measurement is from 88 mm. to 100 mm.

V. The pubic center is present on the 119th day in an embryo with a C.R. length of 133 mm. At this time all other centers which appear, until just prior or subsequent to birth, are usually apparent.

VI. Practically all antenatal pelvic ossification centers are evident by the end of the 19th week of fetal life.

REFERENCES.

1. Keibel and Mall. *Human Embryology.*

2. A. Rambaud and Ch. Renault. Origine et developpement des Os, Paris, 1864.

3. Petersen. Untersuchungen zur Entwickelungsgeschichte des Menschlichen Beckens. *Arch. für Anat.*, 1893, p. 67.

4. P. Bade. Die Entwicklung des menschlichen Skelets bis zur Geburt. Eine röntgenographische Studie. *Archiv f. mik. Anat. u. Entwick.*, 1899-1900, lv, p. 245.

5. W. Hagen. Die Bildung des Knorperskelets beim menschlichen Embryo. *Arch. f. Anat. u. Physiol.* (*Anat. Abteil.*), 1900

6. Lambertz. Die Entwicklung des menschlichen Knochengerüstes während des fötalen Lebens. *Fortschr a. d. Geb. d. Röntgenstrahlen, Ergänzungsheft* I, 1900.

7. F. P. Mall. On Ossification Centers in Human Embryos Less than 100 Days Old. *Amer. Jour. Anat.*, 1905–06, v, 433.

8. Hess. The Diagnosis of the Age of the Fetus by the Use of Röntgenograms. *Amer. Jour. of Dis. of Children.*, 1917, xiv, 397–423.

9. Obata. Die Knochenkerne des fetalen menschlichen Beckens. *Zeitschft. f. Geb. ü. Gynäk.*, 1912, lxxii, 533–574.

Reprinted from The Anatomical Record,
Vol. 14, No. 6, June, 1918

351

No 2.

3. HOW CAN ANATOMY BE TAUGHT AS A SCIENCE AND FULFILL ITS PURPOSE AS A FOUNDATION FOR MEDICINE?

C. M. JACKSON

Institute of Anatomy, University of Minnesota, Minneapolis

It is now generally agreed that in medical schools the fundamental branches should be taught primarily as sciences, but at the same time with a view to their purpose as a foundation for medicine. Thus anatomy on the one hand must furnish a comprehensive and rational explanation of the form and structure of the human body, and on the other hand it must provide a 'working knowledge' as a basis for subsequent study in physiology, pathology, and clinical medicine. This ideal with its twofold purpose is clear. The question is, can it be realized? And, if so, how is it to be done?

The primary purpose—to teach human anatomy as a science—is, at least in theory, not an especially difficult problem. In general, the object is to study the human mechanism in the light of its individual and racial history; to explain the human body as a biological organism. Given a competent teacher, students familiar with the principles of animal morphology, and the facilities of a modern anatomical laboratory, this task could readily be accomplished. It would require merely a carefully planned lecture and laboratory course, including a brief dissection of the human body, with correlated work in histology and embryology.

But this knowledge of the general principles of human morphology, while indispensable, is insufficient for the needs of the medical student. His training must include more. He needs in addition many specific details of human structure which are necessary to solve the problems of physiology, pathology, and clinical medicine. To teach anatomy as a science and at the same time to meet the requirement for specific knowledge in the brief time available is the difficult task which confronts us as teachers of anatomy.

From the clinical point of view, it is desirable that the medical student should know all about human anatomy, since the exact details needed cannot be foreseen. Yet this is clearly impossible. The student might easily spend years in the study of gross anatomy alone, without exhausting the subject.

It is evident that there must be a selection of the subject matter to be taught. The best that can be done is in teaching anatomy as a science to use as illustrative material so far as possible those concrete details most likely to be of use later and to present them in such a way as to give the student a collateral training which will be of maximum value.

To make wisely this selection of subject matter with a view to its practical application later requires of the anatomist a knowledge of the subsequent work in medicine. While it is not necessary for him to be a graduate in medicine, he should at least have a sufficient comprehension of physiology, pathology, and clinical medicine to judge as to what will probably constitute the most useful preparation for these subjects.

Even with the best possible selection of subject material, much anatomy which the student may need must necessarily be omitted. The situation is materially improved, however, if the elective principle is adopted in the medical curriculum. According to this principle, it is frankly recognized that no student can be expected to learn all about every useful subject in the curriculum. He can hope at best merely to get a fair grasp of the fundamental principles in each subject, with such details and training in handling them as will serve his purpose in the more frequently met and important phases of medical science and practice.

By cutting down the various required courses to the bare essentials, the medical curriculum, now overcrowded in most schools, can be somewhat reduced so as to give room for electives. This will permit the individual student to supplement his required courses by further elective work in the lines of his greatest needs, and thus give him a training better than is possible with an inflexible curriculum. While the time which can thus be devoted to electives is necessarily limited, the system is a decided improvement. Although but few medical schools have as yet adopted this principle, many more will doubtless do so when its advantages are better understood.

Our experience with the elective system in the Medical School of the University of Minnesota may be of interest in this connection. In 1914 we reduced our overcrowded and inflexible medical curriculum from a total of about 4,800 hours to 3,624 hours of required work. Throughout the sophomore, junior, and senior years, on two half-days a week no required work is now scheduled, this time being left open for elective courses offered in the various departments. From these elective courses, each student must choose the equivalent of six hours a week, or a total of 576 hours in the three years. Thus about 14 per cent of the curriculum is made up of electives, which may be chosen in any line for which the student is prepared.

In general, the system has worked out well, and is very satisfactory to both students and faculty. As in other departments, the amount of required work in anatomy had to be reduced, in order to give room for electives in the curriculum. By abbreviating some of the courses, and making topographic anatomy elective, the required work in the department of anatomy (including gross anatomy, histology, and embryology) was reduced from 832 hours to 688 hours. This required work is now distributed as follows:

SUBJECT	HOURS	YEAR
Gross anatomy	336	First
Histology	160	First
Embryology	96	First
Neurology	96	Second
Total	688	

Various electives are offered in the department of anatomy, with enrollment during the past year as follows:

Special dissections	32
Topographic anatomy	14
Applied anatomy	14
Advanced anatomy	18
Anatomical drawings	3
Anatomical technique	13
Advanced histology	23
Implantation and placentation	3
Fetal anatomy	13
Special embryology	8
Experimental neurology	5
Research in anatomy	10
Anatomical seminar	8
Total enrollment	164

These electives vary in time, the average occupying about 80 hours. Thus, the total of the electives taken in anatomy amounts to an average of nearly 170 hours for each student in a class of 75. This would bring the total average per student up to about 858 hours (required and elective), or slightly more than that formerly required of every student. Even though the total amount of time devoted to anatomy were the same, the efficiency of the instruction would be increased, because the work is distributed more in accordance with the varying needs and capabilities of the individual student.

The elective system requires more work by the staff, for it is evident that to teach several shorter, more specialized courses to students in small groups will require more work than to teach an equivalent amount of routine class work to students in larger groups. But the results justify the increased work by the staff, and the elective courses are profitable to the teachers themselves as well as to the students.

The students who choose their elective work in anatomy do so for various reasons. A few (would there were more!) take advanced anatomy because they become interested in the subject for its own sake or as a possible career. The majority elect it because they realize the advantage of a more extensive training in anatomy as a foundation for clinical work. Some have in mind a better preparation for definite clinical fields in which they hope later to specialize. Whatever their purpose, the elective system permits them to obtain a training in anatomy far better than would be possible under the inflexible required system.

Another phase of increasing importance in medical education which concerns anatomy is the graduate work. The modern university must meet the growing demand for trained specialists in the various fields. In this connection, anatomy is concerned, 1) in the training of anatomists themselves, as recruits to the profession and, 2) in contributing to the training of specialists in other fields. As a major line of graduate work, anatomy should be open to candidates for the Master's or Doctor's (Ph.D.) degree in the graduate school on the same basis as any other scientific subject. Teaching fellowships should be provided which will help to defray the student's expenses, while the experience in teaching will probably be of great value to him in his future career. The training of recruits for the profession of anatomical teaching and research is an important phase of our work which deserves careful consideration.

The most recent phase of graduate medical education is in the clinical branches. Even under the elective system it is a recognized impossibility to provide adequate training for specialists in the various departments of medicine and surgery in the undergraduate curriculum. For the adequate training of such specialists, at least three years of graduate work are now generally considered essential, and the universities are beginning to make definite provision for the establishment of such courses. In this new field of graduate medical education, anatomy is also directly concerned, as is evident when the requirements for adequate training in clinical subjects are considered.

In graduate clinical work, as in graduate work generally, no definite and fixed curriculum can be formulated, the work being advanced in character and adapted to the individual student. Certain general principles are self-evident, however, and should be kept clearly in mind in planning the work in each case. It must be remembered that in anatomy, as in the other fundamental sciences, the training of the average medical student (as we have seen) has necessarily been limited to that which may suffice for purposes of general practice. If he desires to specialize in any clinical branch, he must strengthen his foundation by further work in the fundamentals, otherwise the clinical superstructure will be unsatisfactory. Just as anatomy, physiology, and pathology form the foundation upon which the undergraduate clinical training is supported, so must more extensive work in these fundamentals form the basis for any efficient system of graduate training in specialized clinical lines.

We have had considerable experience at Minnesota during the past few years in the development of graduate work in the clinical subjects, and it may be of interest to know how it works out with reference to anatomy. As might be expected, those specializing in the various phases of internal medicine require, as a rule, comparatively little additional work in anatomy. In surgery and allied subjects, however, the graduate students find it profitable to spend a considerable amount of time on advanced work in anatomy. They frequently make anatomy their minor department in working for advanced clinical degrees.

In ophthalmology and oto-laryngology, for example, the graduate students usually take advanced work in anatomy for two half-days a week during the first year. In the first semester this work is in gross anatomy, consisting of 1) a review of the skull, with intensive study of special preparations; 2) topographic anatomy of the head and neck, studied especially by sections in the three planes, and, 3) special dissections for the regional anatomy of the eye, ear, nose, and throat. This is followed in the second semester by a similar study of the special histology and embryology of the eye, ear, nose, and throat, including gross fetal dissections and histological preparations of these regions in the human embryo and adult. Those making anatomy a minor are required to take additional work in some appropriate phase of the subject. For example, the wax-cast model of the adult nasal cavity and paranasal sinuses (which is demonstrated at this meeting) was constructed by Dr. Connor as a part of his work for a minor in anatomy.

Thus it is evident that anatomy must play a rôle of increasing importance in the development of graduate work, not only in the training of professional anatomists, but also in the training of clinical specialists in the various branches. In these new fields of anatomical work, as well as in the older and more familiar lines, the question of methods of teaching is one of perennial interest. Lack of time prohibits me from considering pedagogical methods, aside from a few general principles which may be briefly mentioned.

In the first place, uniformity of methods in teaching anatomy is unnecessary and even undesirable. The methods would naturally vary according to the preparation and ability of the students, the facilities available, and the individuality of the teacher. To be efficient, however, they should regard the past, the present, and the future. As to the past, the instruction in anatomy should of course be closely related to the previous work in zoology. As to the present, every effort should be made to correlate the work in gross anatomy with that in histology and embryology. And the future applications should be kept constantly in mind, as has already been emphasized.

The physiological view-point is one which is often very useful in teaching anatomy, even though we may not go so far as to admit that "'anatomy without physiology is like an old maid without a dowry." In reviewing the locomotor apparatus, for example, I have found it a stimulating exercise for the students to reason out what symptoms would be expected if a given nerve trunk were cut and to give a complete explanation of the various structures involved. In the case of a mixed nerve, such as the femoral, this would involve both sensory and motor symptoms. The explanation of the sensory symptoms would include the distribution of the various sensory branches to corresponding areas of partial or complete anesthesia in the integument, joints, and muscles. The explanation of the motor symptoms would include, in the first place, the posture of the part, due to the unopposed action of the unparalyzed muscles. In the second place, it would include the movements which would be weakened or lost, due to partial or complete paralysis of the muscle groups innervated by the various branches of the nerve. Such an explanation thus involves a review of the anatomy of the nerves, muscles, bones, and joints in considerable detail. But it does more in that it helps to organize that knowledge into a workable form of obvious value later not only for physiology, but also for clinical medicine. In general, in our methods of teaching we should always bear in mind that, as ex-President Eliot has said, professional training is primarily not for information, but for power.

To summarize briefly: human anatomy can be taught as a science, and must be if it is to be efficient. By a judicious choice of methods and selection of subject matter, it can also fulfill its purpose as a foundation for medicine. The difficulties due to the limitation of time may be largely overcome by the adoption in the medical curriculum of the elective principle, permitting students to take supplementary work in anatomy corresponding to their individual needs. Graduate courses in anatomy should also be provided for those desiring to specialize further, either in anatomy or in other medical subjects. The methods of instruction in anatomy may vary, but they should always have due regard for the preparation of the student, should correlate closely the various phases of the work, and should develop a 'working knowledge' which will serve as a basis for future application.

0

No. 3.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MARCH 17

Reprinted from THE AMERICAN JOURNAL OF
ANATOMY, Vol. 25, No. 3, May, 1919

THE POSTNATAL DEVELOPMENT OF THE SUPRARENAL GLAND—AND THE EFFECTS OF INANITION UPON ITS GROWTH AND STRUCTURE IN THE ALBINO RAT

C. M. JACKSON

Institute of Anatomy, University of Minnesota, Minneapolis

TEN FIGURES

CONTENTS

Material and methods.......... 222
Volumes of suprarenal cortex and medulla.......... 228
Volumes of suprarenal parenchyma and stroma.......... 232
Size of parenchyma cells and nuclei.......... 233
Cell division—frequency of mitosis.......... 239
Histological changes in the suprarenal gland.......... 244
  1. Normal postnatal histogenesis.......... 244
  2. Changes in young rats stunted by underfeeding.......... 261
  3. Changes in rats refed after stunting by underfeeding.......... 263
  4. Changes in adult rats after acute or chronic inanition.......... 264
Morphogenesis of the suprarenal gland.......... 269
Summary.......... 270
Literature cited.......... 277

In a previous paper (Jackson, '13) the relative growth curve of the suprarenal gland in the albino rat was described, including the characteristic difference in weight according to sex. In later papers (Jackson, '15 a, '15 b) the changes in the weights of the suprarenal in underfed young rats, and in adult rats during acute and chronic inanition were considered. The object of the present study is to extend these observations so as to include the volumetric analysis and histological structure of the suprarenal cortex and medulla during normal postnatal development and as affected by inanition and by refeeding.

This paper forms the fifth of a series of studies upon the effects of inanition in the albino rat, the investigation being supported by a special grant from the research funds of the Graduate School of the University of Minnesota.

MATERIAL AND METHODS

The material used included the suprarenals from 108 albino rats (Mus norvegicus albinus), obtained partly in connection with my previous studies ('15 a and '15 b), partly, from material collected by Hoskins ('16) and Stewart ('16), and partly from new material. The 108 rats include 52 normal (control) rats of both sexes, varying from new-born to about one year of age; 16 young rats held nearly at maintenance (constant body weight) or severely stunted by underfeeding for various periods; 10 rats refed after such underfeeding and 1 refed after acute ananition (adult); 13 adult rats subjected to acute inanition; and 6 subjected to chronic inanition (table 1).

The diet in all cases was whole-wheat (graham) bread soaked in whole milk, the amount being reduced during the maintenance and chronic inanition experiments and cut off entirely in acute inanition. Water ad libitum was supplied in all cases. In the chronic-inanition experiments the amount of food was gradually reduced through a period of about five weeks. The percentage losses and final body weights are given in tables 1 D and 1 E.

In order to keep the animals alive during the longer experimental periods, especially in the case of the young rats underfed for long periods, it was found necessary to keep them in a warm room. The cages were provided with wire-net bottoms to prevent the rats from eating their feces.

The general data for the individual rats used are shown in table 1. In the first column the letters indicate the series to which the rats belong. The number preceding the decimal point is the litter number and the number following designates the individual rat. In several of the rats used for adult acute- and chronic-inanition tests, however, the litter records were not available. The sex, age (where known), nose-anus length, gross body weight (in some cases also net, without intestinal contents), and the fresh weight of both suprarenals are recorded in the table.

In the subsequent tables 2, 3, and 5, the data are usually grouped for economy of space. The individual data will be filed in The Wistar Institute of Anatomy, Philadelphia, where they may be consulted by those interested.

The suprarenals were removed immediately after the animals had been killed. The rats were killed by chloroform, excepting those used for the chromaffin tests (series F), which were killed by cutting their throats. One rat (F 6.2) died from acute inanition.

In most cases the glands were fixed in Zenker's fluid, embedded in paraffin, cut in serial sections (3 μ to 5 μ), and stained with haematoxylin and eosin. In a few cases other fixatives (formalin, Zenker-formol, Flemming's) and other stains (iron-haematoxylin, Mallory's anilin-blue connective-tissue stain, etc.) were employed. In the series F and a few others one gland was immediately placed in Müller's fluid for one week, then sectioned with the freezing microtome. The sections were mounted in glycerin for the study of the chromaffin reaction. The other gland was first fixed one or two hours in 10 per cent aqueous formalin solution (neutralized with lithium carbonate). In a few cases the glands were left in 5 per cent formalin from eighteen to twenty-four hours, without apparent effect upon the liposomes. Bell ('10), however, found in some cases a partial disappearance of the liposomes in muscle and epithelium after even a brief exposure to formalin. The formalin-hardened gland was cut with the freezing microtome into sections (at 10 μ).

Some of these sections were placed for about twenty hours in 1 per cent aqueous osmic acid solution, and the others stained with scarlet red and mounted in glycerin for special study of the lipoids and fat. Herxheimer's formula (saturated solution of scarlet red in 70 per cent alcohol containing 2 per cent of sodium hydroxide) was used. The sections were first placed in 60 per cent alcohol (one minute), then in Herxheimer's fluid (three minutes), then rinsed in 60 per cent alcohol (one-half minute), washed in distilled water a few minutes, and mounted in glycerin.

Frozen sections were also studied fresh, mounted in normal salt solution, and also treated with 1 per cent potassium hydroxide, 1 per cent acetic acid, alcohol, etc. The frozen sections were cut by means of a Spencer automatic freezing microtome, using liquid carbon dioxide for refrigeration.

The method used for the volumetric determinations is a modification of Hammar's paper method, as described in my work on the hypophysis (Jackson, '17). The outlines of the magnified sections are projected and drawn upon 'American linen record' paper (sheets 18 x 23 inches, 36 lbs. per ream) by means of an Edinger projection apparatus. Four samples, each 5 cm. square, are weighed from each sheet, and the area corresponding to each gram of paper determined. The structures whose volume is to be determined are then cut out and weighed, and the corresponding area calculated. This magnified area is then reduced to actual area (dividing by the square of the magnification). Multiplying by the actual thickness of the sections now gives the actual volume of the parts concerned. For determining the volumes of cortex and medulla (table 2) it was not considered necessary to draw every section. Enough were taken to make a total of at least fifty drawings for each suprarenal. This required every fourth section for some of the smaller glands, but fewer for the larger glands, some of the largest requiring only every sixteenth section. A magnification of 75 diameters was used for the cortex and medulla.

As was found in the hypophysis, the volumes obtained (in cubic centimeters) are usually considerably less than half the corresponding fresh weight of the gland (in grams). The difference is due: 1) to the density of the gland; 2) to the fibrous capsule and attached extracapsular tissue, weighed but not measured (forming about 11 per cent of the gland in the dog, according to Flint's data), and 3) to the great shrinkage due to the process of fixation, dehydration, and embedding in paraffin.

For determining the relative volumes of parenchyma and stroma (including blood-vessels), a higher magnification was required. A Leitz ocular no. 4 and objective no. 7a were used, with large Spencer camera lucida, giving at table level a magnification of about 500 diameters. As it is impracticable to measure the entire gland in this way, typical areas are selected and drawn from the various regions of the cortex and medulla, outlining merely the areas of parenchyma and vascular stroma in a given plane. These areas are then cut out of the paper and weighed. Since it is desired merely to determine the relative proportions of parenchyma versus stroma, it is necessary merely to compare the weights of the paper representing the corresponding areas. In this way the results summarized in table 3 were obtained. These results are of course merely approximate, based upon the assumption that the areas selected represent the typical or average condition for the entire gland.

For the volumetric determinations on parenchyma cells and nuclei (table 4) a similar plan was used. In this case a Zeiss 2-mm. apochromatic objective with ocular no. 4 was used, giving at table level a magnification of about 1600 diameters. Typical fields were selected and drawn with the camera lucida, outlining parenchyma cells and nuclei (stroma and vessels omitted) in the various zones of the cortex and medulla. No change of focus is permissible while the drawing is being made, since the volumetric calculations are based upon the assumption that it represents a true optical plane.

The further procedure is as follows: The paper representing the areas drawn from the various zones is weighed (to the milligram). Then the nuclei are cut out with a sharp-pointed scalpel, the paper resting upon a wax plate. The paper representing the nuclear areas and the remainder (cytoplasm) are then weighed separately. From the weight of the nuclear areas, the magnified area in square centimeters is calculated. This divided by the square of the magnification and by the number of nuclei involved gives the average actual area per nucleus. If we assume that the nuclei are spherical and not greatly different in size, the average area per nucleus in a given optical plane should, according to the rules of solid geometry, represent two-thirds the area of the corresponding great circle.

This result is derived as follows: The volume of any sphere equals two-thirds the volume of the circumscribed cylinder. The diameter of the sphere equals the height of this circumscribed cylinder, and the area of the great circle of the sphere equals the cross-sectional area of the cylinder. The volume of the cylinder is obtained by the product of its cross-sectional area × height of cylinder. The volume of the sphere may be obtained by the product of the average cross-sectional area (i.e., the average of a series of parallel planes, perpendicular to any diameter) of the sphere × the diameter of the sphere. Since the height of the cylinder equals the diameter of the sphere, the average cross-sectional area of the sphere must equal two-thirds of the cross-sectional area of the cylinder (or of the great circle area of the sphere), since the corresponding products (the volumes of the sphere and the circumscribed cylinder) bear that ratio.

Hence in an optical plane, as seen in a histological section, assuming the nuclei to be approximately spherical and similar in size, the observed average nuclear area should correspond approximately to two-thirds the corresponding great circle area of the nucleus. From the great circle area ($= \pi r^2$), the average nuclear diameter ($= 2 r$) and volume ($= 4/3 \pi r^3$) are readily calculated.

The average nuclear dimensions being known, the average total cell dimensions may also be calculated, assuming that the cytoplasm surrounds the nucleus in a uniform layer, making the whole cell approximately spherical.

Another relation, which may not appear evident at first glance, is that in the optical plane of any section the sum total of the nuclear area bears the same ratio to the sum total of the cell areas as does the volume of the average nucleus to the volume of the corresponding average cell. This relation is proved as follows, assuming the cells to be fairly uniform: The volume of any given region is made up of its parenchyma cells (stroma being excluded). The ratio of this volume, the total cell volume, to the total nuclear volume is the same as that of the average cell volume to the average nuclear volume (since the average volume is derived from the total by dividing by the total number of cells (or nuclei) in the region). But the total cell volume and total nuclear volume for a region may be derived in another way. The region may be considered as made up of parallel sections of minimal thickness, as found in histological preparations. In each section, the total cell volume is equal to the total area of the region measured (stroma excluded) × the thickness of the section; and the total nuclear volume likewise corresponds to the sum total of the nuclear areas × the thickness of the section. The total cell and nuclear volumes for the region would be obtained by multiplying by the total number of sections. Thus it is evident that the ratio of total cell volume to total nuclear volume of a region (or of average cell volume to average nuclear volume) is the same as the ratio of the total area to the total nuclear area in a typical optical plane. The total area of a section is represented by the total paper weight for that section and the total nuclear area by the paper weight of the nuclear areas. Moreover, the ratio of the average cell diameter to the corresponding nuclear diameter is the same as the ratio of the cube roots of the average cell volume and nuclear volume. Therefore, for a given region, assuming that the cells are fairly uniform spheres, the average

$$\text{Cell diameter} = \text{Nuclear diameter} \times \sqrt[3]{\frac{\text{Total paper weight}}{\text{weight of nuclear areas}}}$$

From the cell diameter, the cell volume is readily obtained, and the volume of cytoplasm in this average cell will be the difference between the cell volume and the nuclear volume. The results are given in table 4. Errors in computation[1] may be checked by comparing the ratio of nuclear volume and cell volume with the corresponding ratio of the cube of the nuclear diameter and the cube of the cell diameter. Crelle's Rechentafeln were found helpful in the calculations.

The mitoses were counted in each case in an entire section through the middle of the gland. A 2-mm. Zeiss apochromatic objective and a mechanical stage were used. The results are summarized in table 5.

---

[1] In my previous paper on the hypophysis (Jackson, '17) an unfortunate error was made in the calculation of the parenchyma cell diameters listed in the last column of table 3, p. 333. Instead of 10.1, 11.9, 13.6, 9.7, 10.2, 10.0, 10.8, and 11.0, this column should read: 8.4, 9.3, 10.4, 7.8, 8.0, 8.1, 8.6, and 8.7. A corresponding correction is necessary for cell diameters on the lower part of page 335, and in the Summary, paragraph 7, page 355, of the same paper. A similar correction for the diameter of the parenchyma cells should be made in the abstract of that paper, published in the Anatomical Record, vol. 11, p. 370, lines 5 to 8.

VOLUMES OF SUPRARENAL CORTEX AND MEDULLA

Volumetric data for the relative volumes of the suprarenal cortex and medulla are given in table 2. These will be considered successively, 1) during normal postnatal development; 2) in young rats stunted by underfeeding; 3) in such rats refed after stunting, and 4) in adult rats subjected to acute or chronic inanition.

1. During normal postnatal development

The data summarized in table 2 A include the relative volumes of cortex and medulla in groups at various ages, beginning at seven days. (In the new-born the cortex and medulla are still intermingled and were not measured.) If the data for the absolute volumes (not given in the table) are considered, it appears that both cortex and medulla continue to grow from birth to one year of age. Although there are marked individual variations, it is on the whole clearly evident that the cortex and medulla grow at different rates, so that their relative sizes change. During the second and third weeks, the cortex grows more rapidly, increasing from 75 or 80 per cent to about 90 per cent of the entire gland, by volume. It apparently continues to increase relatively, averaging about 93 per cent at ten weeks of age. In older rats, there are marked individual variations, but the cortex appears to decrease slightly to 90 or 91 per cent in average relative volume.

The medulla increases more slowly in absolute size, so that its relative volume decreases (inversely with the cortex). The medulla is relatively small in the new-born, though difficult to measure on account of the intermingled cortex. In connection with the process of confluence, the medulla expands rapidly, being relatively largest at one week, forming 20 to 25 per cent of the total volume. It decreases to about 15 per cent at two weeks, 10 per cent at three weeks, and to about 7 per cent at ten weeks, increasing again slightly to an average of 9 or 10 per cent of the entire suprarenal in the older rats. In a few cases in which the whole suprarenal gland is relatively small, the medulla was found relatively large (as in the female St 5.1 at 56 days). In very large glands, on the contrary, the medulla is usually relatively small. This suggests that the variability in the size of the suprarenal may be due mostly to variability in the volume of the cortex.

On account of the known sexual difference in the weight of the suprarenals (which become relatively heavier in the female from the age of about six weeks),[2] the question naturally arises concerning the relative sizes of cortex and medulla in the two sexes. As shown by table 2 A, however, the present data grouped by sexes reveal no constant or important differences in this respect. A study of the individual data likewise reveals no constant sexual differences in the absolute or relative sizes of the suprarenal cortex and medulla. It is possible that a larger number of observations would reveal differences obscured by large individual variations (or experimental error) in the present small series of data. Provisionally, however, on the basis of the present data, I would conclude that the relatively larger suprarenal gland in the female is due to an increase in both cortex and medulla, with no important change in their relative sizes as compared with those of the smaller gland in the male. Thus the conditions in the suprarenal would appear to be different from those in the hypophysis, where the relatively larger gland in the female is apparently due to a larger pars anterior (Jackson, '17).

In one female (J 1.7) which had just given birth to a litter the suprarenal glands were not hypertrophied (table 1), being on the contrary below the normal weight for corresponding body weight or length. The volumetric relations of cortex and medulla appear normal, the latter forming 7.2 per cent of the total volume. Hypertrophy of the suprarenal gland (especially of the cortex) during pregnancy has been described by various authors.

---

[2] Elliott and Tuckett ('06) noted in several cases that in the rat, cat, guinea-pig, and rabbit of reproductive age the suprarenal glands were larger in the female. They considered this a temporary hypertrophy, however, concluding that "Gestation accelerates the growth of the gland, so that for a time that of the female outstrips the male. In such increase the cortex is concerned, but there is undoubtedly growth of the medulla also. Our analyses are too few to trace the changes satisfactorily." That the larger suprarenal in the female rat is a regular sexual characteristic, independent of gestation, was first established by Hatai ('13) and Jackson ('13).

Elliott and Tuckett ('06) found great variation in the relative volumes of cortex and medulla in the suprarenal gland of adult animals. Thus the medulla varies from 1.6 per cent of the gland in the guinea-pig to 50 per cent in the fowl. In the adult rat the medulla forms about 5 per cent of the gland. In very young mammals the medulla is relatively larger, the growth of the suprarenal after early youth being apparently in the cortex alone. They also noted a relatively larger suprarenal in the female, ascribing this to pregnancy.[3]

Bager ('17) found the suprarenal glands of the rabbit relatively heavier in the female after the age of puberty.

A relative increase in the amount of cortex, with corresponding decrease in the relative size of the medulla in the suprarenal gland according to age, was previously noted by Canalis ('87) in the rabbit, and by Hultgren and Anderson ('99) in various mammals, especially cat and rabbit. Soulié ('03), however, describes the suprarenal medulla as relatively small in the human fetus, forming hardly 10 per cent in the new-born, the proportion being a little higher in the adult and relatively greater in other mammals. Scheel ('08), Starkel and Wegrzynowski ('10), Thomas ('11), and others describe a relative increase in the postnatal human medulla (which will be considered later under 'Morphogenesis of the suprarenal gland'). Gottschau ('83), by comparison of the relative widths of cortex and medulla in sections of the suprarenal, concluded that there is great variability in different mammals. Pfaundler ('92) found the ratio of cortex to medulla in the suprarenal of the horse to be variable, the average being about 4: 1, which is nearly in agreement with the data of Flint ('00) for the dog. In the rabbit, Bager ('17) found the medulla to decrease in relative size from 20.8 per cent of the gland in the new-born to 1.84 per cent at one year. No difference was noted according to sex in the ratio of cortex and medulla.

On the whole, therefore, the evidence would appear to support the conclusion that in general the suprarenal medulla in mammals forms but a small part of the gland. It usually appears relatively large in the new-born, however, decreasing later in relative size on account of more rapid growth in the cortex. In the rat, although the suprarenal becomes relatively larger in the female, there is apparently no constant sexual difference in the relative size of cortex and medulla. The whole question of the changes in relative size of cortex and medulla according to age and sex in various species deserves a more careful and extensive study.

---

[3] See footnote on page 229.

2. *In young rats stunted by underfeeding*

As previously shown (Jackson, '15 b), in young rats held at maintenance (nearly constant body weight) by underfeeding for various periods there is a definite tendency to increase in the weight of the suprarenal gland, especially in the female. The observations in table 2 B indicate no marked change in the relative volumes of the cortex and medulla as a result of such experiments, however. While during normal growth up to ten weeks the suprarenal medulla tends to lag behind (cortex becoming relatively larger), in the maintenance experiments it apparently remains nearly constant in relative size. Lewis and Pappenheimer ('16) likewise found no constant change in the relative size of cortex and medulla in the suprarenal glands of children emaciated through malnutrition.

3. *In rats refed after stunting*

As shown by table 2 B, in rats refed one or two weeks (after being held at maintenance from three to twelve weeks of age), the relative sizes of the suprarenal cortex and medulla appear nearly normal. In a permanently stunted female (St 33.120, refed to one year after maintenance from three to twenty-one weeks of age), the medulla appears relatively large, while in a male (S 33.118) similarly refed the ratio appears nearly normal. Stewart ('16) found that in rats refed four weeks or less, after maintenance from three to twelve weeks of age, the suprarenals seem to lag behind somewhat in weight, although they appeared normal in such rats refed to the age of one year.

4. In adult rats during acute or chronic inanition

In adult rats subjected to acute inanition seven to nine days, with loss of about one-third in body weight, the relative volumes of cortex and medulla remain nearly normal (table 2 B), indicating that the loss (if any) has been nearly proportionately distributed. In rats after chronic inanition due to gradual decrease of food for five weeks, with loss of about 36 per cent in body weight, the medulla averages a little higher. This may indicate a relatively greater loss of the cortex, but the difference is small and of doubtful significance.

VOLUMES OF SUPRARENAL PARENCHYMA AND STROMA

The averages (and ranges) of the percentages by volume formed by the stroma, including the vessels, are summarized by groups in table 3. The variations are due chiefly to the differences in blood content. On account of the great variability shown, no conclusions can be drawn with certainty. Neither the groups nor the individual data reveal any marked change in the normal vascularity of the gland from new-born to adult. There is in general, however, an increased vascularity evident upon passing from the outer zone of the cortex toward the medulla, with the exception of a decrease in the outer part of the middle zone. More than one-fourth of the medulla by volume is composed of stroma and included vessels.

In the young rats held at maintenance the vascular stroma appears moderately decreased in relative volume in the outer and middle zones and greatly increased in the inner zone, with but little change in the medulla. The hyperemia of the inner zone is usually strikingly apparent in the sections under the microscope (low power).

In the rats refed one or two weeks after maintenance experiments, there appears in general a reduction in the relative volume of the vascular stroma in the most hyperemic regions—medulla and inner cortical zones—and an increase in the vascular stroma of the middle zone. The outer cortical zone, however, appears even more anemic than before.

In adult rats subjected to either acute or chronic inanition, there appears to be an increased vascularity of the middle and inner cortical zones, with a decreased vascularity in the medulla. Marked hyperemia of the suprarenal cortex has also been noted as a result of inanition in the guinea-pig (Martinotti, '92; Rondoni and Montagnani, '15).

The most constant effect of inanition upon the suprarenal stroma is therefore an increased hyperemia of the inner cortical zone. The changes in the vascularity of the remainder of the gland appear variable in different individuals and according to the type of inanition. The variations are due chiefly to the amount of blood remaining in the vessels, which of course may vary greatly under different circumstances. Moreover, the amount retained in the vessels after death may be quite different from that during life. In pathological histology, however, it is customary to judge as to relative anemia or hyperemia by the distention of the vessels in prepared sections of organs. So an attempt to measure the amount appears justified, even though the results are only roughly approximate.

SIZE OF PARENCHYMA CELLS AND NUCLEI

The average sizes of the parenchyma cells and nuclei were not determined by direct measurement, but were calculated by the method previously explained. The data are given in table 4. The cell and nuclear dimensions will be considered, 1) in normal postnatal development; 2) in young rats stunted by underfeeding; 3) in such rats refed after stunting, and 4) in adult rats with acute or chronic inanition.

1. During normal postnatal development

As noted by Canalis ('87) in the rabbit, there is in the rat a general increase in the size of the cells from birth to maturity (table 4). In average diameter, the cells of the outer cortical zone (zona glomerulosa) increase gradually from about 7 μ at birth to about 8 μ at three weeks and to about 9 μ from ten weeks on.

The middle zone (zona fasciculata) cells during the first week average about 9 μ in diameter, being of approximately the same size throughout the zone. In the outer portion of this zone they increase to about 9.6 μ at two and three weeks, increasing to 14 or 15 μ from ten weeks on. The cells in the inner portion of the middle zone appear slightly larger (10.3–10.7 μ) at two and three weeks, but lag behind thereafter, reaching about 12 μ in diameter from ten weeks on.

The inner zone (zona reticularis) cells during the first three weeks are similar in size to those in the adjacent outer part of the middle zone. Later, however, they lag behind, and at ten weeks are about equal in average diameter to those of the outer zone (8–10 μ). In the adult rat, they are in average size the smallest cells of the suprarenal cortex. They do not vary greatly from 9 μ in average diameter throughout postnatal life. The small size of the cells is associated with the atrophic condition of the inner zone, which undergoes continual absorption (as will be discussed later).

The cells of the suprarenal medulla increase steadily from an average diameter of about 8 μ at birth to 10 μ or 11 μ during the second and third weeks. At ten weeks, they average about 13 μ, and in the adult reach an average diameter of nearly 16 μ.

The nuclear growth of the suprarenal parenchyma cells is in general less rapid than that of the cytoplasm, so that with the increasing volume of the cell the nucleus decreases in relative size, forming a steadily decreasing percentage of the average cell volume (table 4). The outer zone nuclei apparently decrease in average diameter from 5.4 μ at birth to 4.6 μ at one week. They increase to about 5.5 μ at three weeks, thereafter remaining nearly constant in absolute size. In relative volume, however, they decrease from 44 per cent of the average cell volume in the new-born to about 23 per cent in the adult rat.

The middle-zone nuclei likewise appear to decrease slightly during the first two weeks (from average diameter of 6.2 μ to 5.6 μ), but increase to 6.2 μ at three weeks. Thereafter the nuclei of the inner part of the zone (like the corresponding cells) increase but little in average diameter, while those of the outer part of the middle zone increase to an average diameter of about 7μ. In relative volume, the nuclei of the outer part of the zone decrease from about 33 per cent of the average cell volume at birth to about 10 per cent in the adult, while the nuclei of the inner part of the zone decrease from about 28 per cent to about 15 per cent in the adult.

The nuclei of the inner cortical zone apparently decrease in average diameter from 6.1μ in the new-born to from 5 to 5.8μ thereafter. In relative volume, they decrease from about 28 per cent of the average cell volume at birth to about 20 per cent thereafter.

The nuclei of the medulla parenchyma cells increase in average diameter from about 6μ at birth to about 6.7μ at three weeks, 7.2μ at ten weeks, and 7.3μ in the adult. In relative volume, they decrease from about 46 per cent of the average cell volume in the new-born to less than 10 per cent in the adult.

Da Costa ('13), in the suprarenal gland of the rat (Mus decumanus), gives the following measurements for the nuclei: outer cortical zone, $4 \times 6 \mu$; middle zone, spherical, 6μ; inner zone, spherical, smaller. My data (previously cited) indicate a somewhat larger average size for the nuclei of the middle zone.

Canalis ('87) noted that, in general, the suprarenal parenchyma cells of both cortex and medulla are larger in the adult than in the young (rabbit, dog, guinea-pig). In the mouse Inaba ('91) found the nuclei of medulla cells increasing in diameter from 5.2μ in the new-born to 6μ at twenty-nine days and later. The cortical nuclei (near the corticomedullary boundary) showed a decrease in diameter from an average of 6.5μ in the new-born to 5μ at twenty-nine days and later. This would indicate that there is an atrophic process of the inner cortical zone in the mouse corresponding to that found in the rat and other forms (to be discussed later).

Mann ('16) noted a seasonal change in the suprarenal cells (both cortex and medulla) of the gopher, which increase in size in the spring, with decrease later and during hibernation.

2. In young rats stunted by underfeeding

Table 4 includes the individual results in four young rats held at maintenance from three weeks to ten or twelve weeks, and one in which the experiment continued to 139 days. Since in the latter case the cells and nuclei are similar in size to those of the other cases, the average is also given for the entire group. In the following discussion the data are compared with those of normal rats at the same body weight (that is, at three weeks, when the experiment began).

The cells of the outer zone have in some cases apparently increased in average diameter, but the average for the group is but slightly increased, if at all. The cells of the outer part of the middle zone show an increased average diameter in all cases, the average for the group being 11.1 μ. This is greater than the normal at three weeks (9.7 μ), though less than the normal at ten weeks (14.5 μ). The cells of the inner part of the middle zone, on the contrary, shows a definite decrease to 8.9 μ (from normal 10.5 μ). The inner-zone cells likewise decrease in average diameter to 8.1 μ (from 9.5 μ), but the cells of the medulla show but little if any change in size.

In nuclear size the outer zone in the suprarenal cortex of the maintenance rats is but slightly changed. The average nuclear diameter is slightly lowered, with a corresponding decrease in the relative volume of the nucleus to 25.7 per cent (from normal 34.5 per cent). The nuclei in the outer part of the middle zone remain nearly unchanged in average diameter, but on account of the increase in cytoplasm and cell volume the relative size of the nucleus decreases to about 17 per cent (from normal 27 per cent) of the cell volume. The nuclei in the inner part of the middle zone show a definite decrease in diameter to 5.5 μ (from normal 6.2 μ), but as the reduction in cell diameter (cytoplasm) is still greater, the relative volume of the nucleus averages slightly higher at 23 per cent of the cell volume (normal 21 per cent). The inner cortical zone nuclei decrease in average diameter to 4.9 μ (from 5.7 μ), but since the cytoplasm decreases similarly, the relative volume of the nucleus is unchanged. The nuclei of the medulla parenchyma cells show a slight decrease in average diameter to 6.4 μ (normal 6.7 μ) and their relative volume decreases to 17.2 per cent (normal 23.5 per cent).

3. In young rats refed after stunting

Table 4 shows the results in four individuals, two refed one week and two refed two weeks after being held at maintenance (nearly constant body weight). In general, the dimensions are slightly greater in those refed two weeks, but as the differences are usually slight, the average is given for the whole group. Comparison is made with the data for the maintenance group and for the normal.

In cell diameter the cells of the outer cortical zone have increased to about normal size (from 8.1 μ at maintenance to 8.7 μ). The cells of the outer part of the middle zone have increased materially (from 11.1 μ maintenance to 11.8 μ after one week of refeeding and to 13.1 μ after two weeks), but are still below the normal. The inner-zone cells have increased to about normal size (9.2 μ). The medulla cells have increased in diameter (from 11.4 at maintenance to 12.2 μ), though still below normal average (13.2 μ).

In nuclear size the outer-zone nuclei remain unchanged in diameter, though decreasing slightly in relative volume (percentage of cell volume). The middle-zone nuclei have not changed much in diameter, but on account of the increase in cell volume their relative volume has decreased to nearly normal ratio. The inner-zone nuclei have increased somewhat in diameter, so the nucleus-plasma ratio is now nearly normal. In the medulla the nuclei have apparently decreased in average diameter (to 6.1 μ as compared with 6.4 μ at maintenance), and this reduces the nuclear percentage of the cell volume from 17.2 per cent to 12.9 per cent, which is considerably below the normal (about 16 per cent). This nuclear change is difficult to understand.

4. In adult acute inanition

As shown by table 4, a comparison of the average data from the four adults with those of the normal adult indicates that the cells of the outer cortical zone have decreased little or none in diameter. The middle-zone cells, however, have decreased in diameter very markedly—from 14.8 μ (normal) to 12 μ in the outer part of the zone, and from about 11.5 μ to 9.2 μ in the inner part of the zone. The inner zone cells have decreased but slightly—from 8.7 μ to 8.5 μ. The medulla parenchyma cells have decreased markedly—from 15.9 μ to 12.7 μ in average diameter.

The nuclei of the outer zone indicate no shrinkage, but rather an insignificant increase in average diameter from 5.5 μ to 5.8 μ, with a corresponding slight increase in relative volume (from 23.2 per cent to 27.1 per cent of the average cell volume). The middle-zone nuclei have decreased slightly in diameter (6.9 μ to 6.5 μ in the outer part and 6.4 μ to 5.5 μ in the inner part of the zone). But since the loss in cytoplasm is greater, the relative nuclear volume has increased—from 10.1 per cent to 16.2 per cent in the outer part and from 15.5 per cent to 21.7 per cent the inner part of the zone. The inner-zone nuclei remain nearly constant in size and relative volume. The medulla nuclei appear slightly decreased in average diameter (7.3 μ to 7.0 μ). But on account of greater loss in the cytoplasm, the relative size of the nuclei has increased from 9.6 per cent to 16.8 per cent of the cell volume.

Barbèra and Bicci ('00), in rabbits starved seven days with loss of 34 per cent in body weight, found the nuclei in the suprarenal cortex to decrease from an average diameter of 7.238 μ to 4.817 μ, and in the suprarenal medulla from 7.773 μ to 5.587 μ. The loss in cytoplasm appeared relatively greater than in the nucleus. Similar results were obtained in dogs. These decreases in cell dimensions would appear to be much greater than those observed by me in the rat. Traina ('04) noted a decrease in the size of cortical cells in the suprarenal of the rabbit during starvation, but gives no exact data.

5. In adult chronic inanition

The data from the two individuals (table 4) indicate that the suprarenal cells of the outer and middle cortical zones have lost in diameter somewhat more than during acute inanition; the inner zone and medulla cells about the same as during acute inanition (or slightly less).

The nuclei in all the regions during chronic inanition have apparently lost in average diameter somewhat more than during acute inanition. The relative (percentage) volume of the nucleus is therefore usually lower than during acute inanition. In the outer zone and the medulla, the nucleus-plasma ratio is not greatly different from that of the normal animal.

CELL DIVISION—FREQUENCY OF MITOSIS

No conclusive evidence of amitosis was found in any of the suprarenal glands studied, although irregular or bilobed nuclei, which might easily be so interpreted, were occasionally observed in the various zones of the cortex and medulla. If amitosis occurs at all (in the rat), it is probably a degenerative phenomenon of no especial significance or importance in the normal development of the gland.

The frequency of mitosis in the suprarenal, under normal and abnormal conditions at various ages, is given in table 5. The average number of mitoses counted in entire sections through the center of the gland is shown for the various zones in the rats grouped according to age or condition.

1. Mitosis during normal development

As shown in table 5 A, the average number of mitoses in an entire section of the suprarenal is somewhat variable in individual cases, but the general trend according to age is evident. The average number per section at birth is 20, decreasing to an average of 10 at one week, and increasing again during the second and third weeks. At twelve days (one case only) there were 40 mitoses per section, which was the maximum number observed, excepting one of the nine cases at three weeks. The average number of mitoses again decreases somewhat slowly from 27 at 14 days to 24 at 21 days, 11 at 56 days, 4 at 67 to 94 days, 2 at 112 to 138 days, and 1 at 340 days. In other adults no mitoses were observed in the suprarenal.

The relative rate of mitosis ('mitotic index' of Minot) decreases more rapidly than is indicated by the figures for the absolute frequency per section shown in table 5 A. This is because the size of the gland, and consequently the total number of cells in a section, is progressively increasing. Thus between birth and three weeks of age the suprarenal gland of the albino rat has increased about four times in weight (and volume). This corresponds to an increase in diameter of about 1.6, or of about 2.6 times in the area of a cross-section. Since the size of the cells has not greatly increased during this time (table 4), the number of cells in a corresponding section of the gland is probably twice as great at three weeks as in the new-born. Thus the relative rate of mitosis would be obtained by comparing the number of mitoses in a section at birth (20) with half the number in a section at three weeks (half of 24 = 12). Therefore, although the absolute number of mitoses per section increases in the second and third weeks, it is doubtful whether the relative rate of mitosis exceeds that of the new-born.

The distribution of the mitoses in the various zones of the cortex and the medulla is also shown in table 5 A. The rate in the medulla is in general nearly parallel with that for the whole gland, decreasing from an average of 6 per section in the new-born to 3 at one week, and gradually increasing again to 6 at fourteen days. Thereafter the number of mitoses decreases, and none are found in the medulla after eight weeks, though continuing to occur in the cortex. The later growth of the medulla must therefore be due entirely to the growth in the preëxisting cells, which continue to increase in average size (table 4).

As to localization in the various regions of the cortex, the number of mitoses in the outer zone (glomerulosa) is strikingly large, although this zone is the narrowest of the three. When it is further considered that the majority of the middle-zone mitoses occur in the outer part of the zone, it is evident that in general the outer portion of the cortex is the chief cortical germinative region. Some mitoses are found scattered throughout the cortex, however, an occasional one being found even in the inner zone (reticularis). In general, mitoses disappear first in the medulla, and the inner cortical zone, and later in the middle zone, persisting longest in the outer cortical zone.

The occurrence of amitosis in the suprarenal has been claimed by various investigators. Mulon ('03 b, '05 a) in particular even maintains that in the guinea-pig amitosis is the predominant type of cell division in the suprarenal, mitosis being much less frequent. Kolmer ('12 b), on the contrary, found mitoses more frequent (occasional amitosis) in the suprarenal cortex of the younger guinea-pigs, though in older, non-pregnant females amitosis was observed with no mitosis. Most authors have considered amitosis in the suprarenal (as elsewhere) to be atypical and degenerative in character. Thus Poll ('99) observed amitosis (more rarely mitosis) in the degenerating cortical cells in transplanted suprarenal glands of the rat. Delamare ('03) observed occasional amitosis in the senile human suprarenal cortex, producing multinucleated masses. Bonnamour ('05 a) believes that the figures which have been interpreted as amitosis (direct division), especially in the outer cortical zone and in the medulla, are merely deformed nuclei, without significance for cell division.

In relation to age, cell division (especially mitosis) in the suprarenal has generally been found more frequent in young animals, as might be expected, and especially in fetal stages (Canalis). It has also been observed in adults, especially in the outer cortical region, by Canalis ('87) in the dog, guinea-pig, rabbit, and mule, Mulon ('03 b) (guinea-pig), Kolmer ('12 b) (guinea-pig), and others. Da Costa('13) has observed mitoses in the cortex of both young and adult dog and guinea-pig.

It is interesting to note that the rate of mitosis in the young rat corresponds rather closely to the growth of the gland in absolute and relative weight. As previously shown (Jackson, '13), the suprarenal gland in the albino rat increases but slightly in average absolute weight (from 0.0019 gram to 0.0023 gram) during the first week after birth, thereby decreasing in relative weight (percentage of the entire body). This is in agreement with the smaller size of the cortical cells (already mentioned) and the corresponding marked decrease in the rate of mitosis observed in the present study (table 5). The weight of the gland increases rapidly during the second and third weeks, reaching its maximun relative size corresponding to the period when an increase in the number of mitoses appears. The formula for the growth of the suprarenal in the albino rat as derived by Hatai ('13) similarly indicates a maximum relative weight for suprarenal at about 10 to 15 grams body weight. His data, however, do not show the arrested growth during the first week. The suprarenal weights in Donaldson's ('15) tables (which are based upon Hatai's formulas) are therefore apparently too high for this early period. According to these tables, at a body weight of 10 grams, the suprarenals should weigh about 0.0045 gram, whereas in fifty-seven cases (30 m., 27 f.) I found an average of only about 0.0023 gram.

As to regional distribution, cell division has in general been found to occur throughout the gland in the earlier stages, especially prenatal (Canalis, '87, in dog, guinea-pig, and rabbit), with a progressive tendency to be restricted later to the outer cortical region (Canalis, '87; Mulon, '03 b, and others). The occurrence of mitosis in the deeper layers of the suprarenal cortex (occasionally even in the zona reticularis) in growing or adult animals has also been observed by Bonnamour ('05 a), Kolmer ('12 b), and Da Costa ('13) (dog, rat, rabbit, guinea-pig). An extraordinary increase in mitosis in the outer part of the zona fasciculata was noted by Kolmer ('12 a) at the end of pregnancy in the guinea-pig. That the outer cortical region of the suprarenal is the chief 'germinative zone' from which by cell division the remainder of the cortex is derived (especially in postnatal development), is indicated by the observations of Canalis ('87), Roud ('02), Cristiani ('02), Soulié (03), Mulon ('03 b, '05 a), Kern ('11), Kolmer ('12 b), Cottentot, Mulon and Zimmerman ('12), and others. Gottschau ('83) considered the germinative zone to lie between the zona glomerulosa and the zona fasciculata, while Wiesel ('01) (in the fetal pig) and Da Costa ('13) placed it in the middle zone (fasciculata), and Dsershinsay (cited by Da Costa, '13) even in the inner zone. Graham ('16) found that after toxic lesions regeneration of the suprarenal cortex proceeds from the zona glomerulosa and outer part of the fasciculata. In the child, mitoses may occur throughout the cortex.

2. Mitosis during inanition and refeeding

From the data given in table 5 B it is evident that the effect of underfeeding in young rats held at maintenance (nearly constant body weight) is in general to diminish very greatly the number of mitoses, and ultimately to prevent them entirely. In rat St 80.9 underfed from birth to twelve days (body weight increased from about 5 grams to 8.9 grams), the total number of mitoses found in a section of the suprarenal was 4, whereas normally (at corresponding age or body weight) it would be between 10 and 20. In ten rats held at maintenance from three to ten weeks of age, the average number of mitoses was reduced to 1, the maximun being 3, and none present in six of the ten cases. In three cases in which the maintenance was continued to the age of twelve to twenty weeks, no mitoses were found in the suprarenal.

The distribution of the mitoses in the various cortical zones of the suprarenal in the stunted rats appears similar to that in the normal glands. That is, the larger number occurs in the outer zone and the outer part of the middle zone. None was noted in the inner zone and (excepting the youngest rat) none in the medulla.

In the rats refed fully for one week (after maintenance from three to twelve weeks) mitosis in the suprarenal has recommenced. The rate is still below normal, however, the average number being only 2 per section. In rats refed two weeks, the number of mitoses has increased to 10 per section, which is nearly the same as that found in the normal rat at fifty-six days. Since the body weight (77 to 79 grams) and suprarenal weight are also nearly the same as in the normal at fifty-six days, we may conclude that the normal rate of mitosis has been reëstablished in the suprarenal glands of stunted rats after two weeks of refeeding. As might be expected, no mitoses were found in the rat refed one year, which had reached maturity.

Martinotti ('92) briefly states that in guinea-pigs starved three or four days an abnormal increase in the number of mitoses (20 to 25) in the suprarenal cortex was always noted. An increase in mitosis was also noted upon refeeding after several days of inanition. Unfortunately, further details are omitted, including the age and number of animals used. Bonnamour ('05 b), on the contrary, found no mitoses in the suprarenal glands of the rat, guinea-pig, rabbit, and cat starved to death. Rondoni and Montagnani ('15) likewise observed no mitosis in the suprarenals in starved guinea-pigs. This would agree with my results on the albino rat.

HISTOLOGICAL CHANGES IN THE SUPRARENAL GLAND

In addition to the volumetric data previously given, the general changes in histological structure were observed. These include: 1) normal postnatal histogenesis in the albino rat; 2) changes in young rats stunted by underfeeding; 3) changes in rats refed after stunting by underfeeding, and 4) changes in adult rats after acute or chronic inanition.

1. *Normal postnatal histogenesis*

A brief account of the histology and histogenesis of the suprarenal will be given, with special emphasis upon the structural features of importance in connection with the changes produced by inanition.

New-born and first week. In sections fixed in Zenker's fluid and stained with hematoxylin-eosin, the three cortical zones of the suprarenal in the new-born rat are fairly distinct. Under low magnification they appear as, 1) an outer zone (glomerulosa), narrow and deeply stained; 2) a middle zone (zona fasciculata), broader, with irregular, radial columns, and 3) an inner zone (zona reticularis), less distinct an more irregular, and (in the new-born) intermingled with the medulla. The medulla, which at birth is not clearly differentiated and separated from the cortex, is more vascular. It is composed of irregular cell groups or cell cords and contains a few scattered, deeply stained cell masses. Similar masses also appear occasionally in the cortex, especially in the region of the hilus.

Under higher magnification, the outer cortical zone appears narrow, but variable in width, about six to ten cells deep. The cells are somewhat variable in size and structure. The cytoplasm is scanty, more or less granular, and presents a few fine vacuoles (lipoids.) The nuclei are spherical or ellipsoidal in form, somewhat deeply chromatic and (as previously shown) present frequent mitoses.

The middle-zone cells are larger. The cytoplasm is more abundant, with distinct eosinophile granules and a few (variable number) of lipoidal vacuoles, somewhat more prominent in cells toward the middle of the zone. The nuclei are spherical and larger than those of the outer zone. They are less deeply chromatic, through occasionally pycnotic.

The inner-zone cells resemble those of the adjacent part of the middle zone, with distinctly granular cytoplasm and a few fine vacuoles. Many of the cells are degenerative in appearance, with indistinct cytoplasm and karyolytic or pycnotic nuclei, sometimes fragmented (karyorrhexis). The inner zone cells of the cortex extend into the medulla, and are intermingled closely with groups of the medulla cells, from which they are frequently difficult to distinguish (in the usual preparations). Inaba ('91) likewise found an intermingling of cortex and medulla in the new-born mouse.

The medulla cells of the suprarenal in the rat are polymorphic, in irregular cords and clumps, separated by relatively large blood sinuses. The cytoplasm is very scanty and the cell boundaries are usually indistinguishable (as also in the cortex). The cytoplasmic (chromaffin?) granules usually appear basophilic, with a bluish-violet stain, in contrast with the typical reddish (eosinophile) color in the cytoplasm of the cortical cells. The nuclei appear similar to those of the adjacent cortex. The stroma is not clearly differentiated. The deeply staining cell clusters above referred to are apparently in most cases remnants of the embryonic sympatho-chromaffin cells, consisting of deeply staining nuclei with scanty cytoplasm. They are distinct from the intermingled cortical cell strands, which are especially evident in preparations stained for lipoids or chromaffin reaction.

The chromaffin reaction is present, but faint, in the new-born rat and increases but slightly in intensity during the first week. The medulla cells assume a pale brownish color, by which they may be clearly distinguished from the cortex and from the intermingled cortical cord present in the medulla. In mammals generally the chromaffin reaction of the medullary cells appears during the fetal period (compare Poll, '05) and was noted by Soulié ('03) well marked in the new-born rat and guinea-pig.

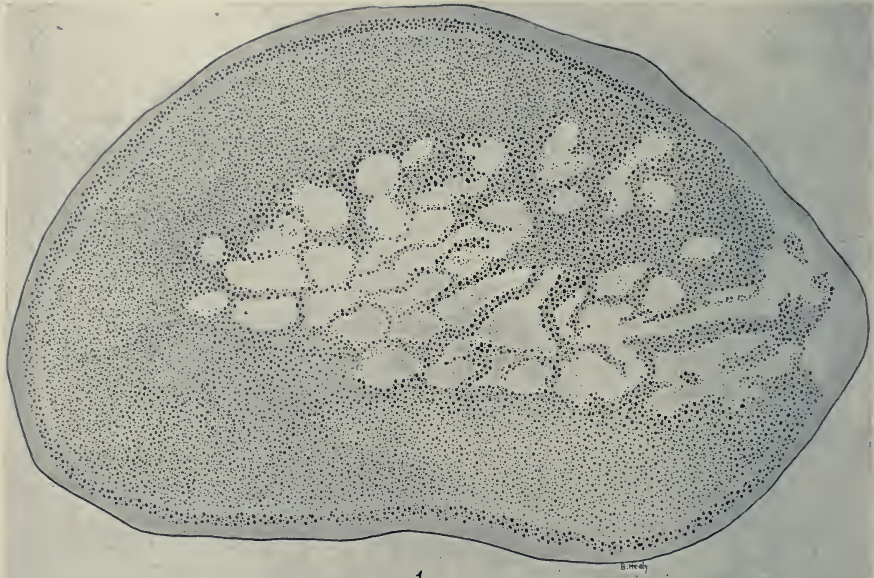
Lipoids. In unstained frozen sections of the suprarenal (formalin-fixed a few hours) in the new-born rat, the cortex appears opaque, due to the emulsion formed by the lipoidal granules. Following the terminology of Albrecht and Bell, these lipoidal granules and droplets will be designated as 'liposomes.' They are soluble in absolute alcohol and xylol, but insoluble in 1 per cent aqueous potassium hydroxide and in 1 per cent acetic acid. With the exception of an indistinct clear streak between the outer and middle zones, they appear somewhat uniformly distributed throughout the cortex and in the cortical cell strands extending throughout the medulla.

These sections, when stained with Herxheimer's scarlet red or with 1 per cent osmic acid and mounted in glycerin, reveal still more clearly the liposomes (fig. 1). Although not so abundant as later, the lipoids appear in much larger amount than would be expected from the corresponding lipoidal vacuoles as seen in the usual stained paraffin sections. Their staining reactions already appear distinct from those of the ordinary fat droplets outside of the fibrous capsule, even when these droplets are as small as some of the liposomes. With Herxheimer's scarlet red, the liposomes stain a deep scarlet-reddish color, while the extracapsular ordinary fat droplets assume a characteristic lighter reddish color. With osmic acid, the ordinary extracapsular fat stains jet-black, the suprarenal liposomes stain in varying shades of brown (mostly light brown).

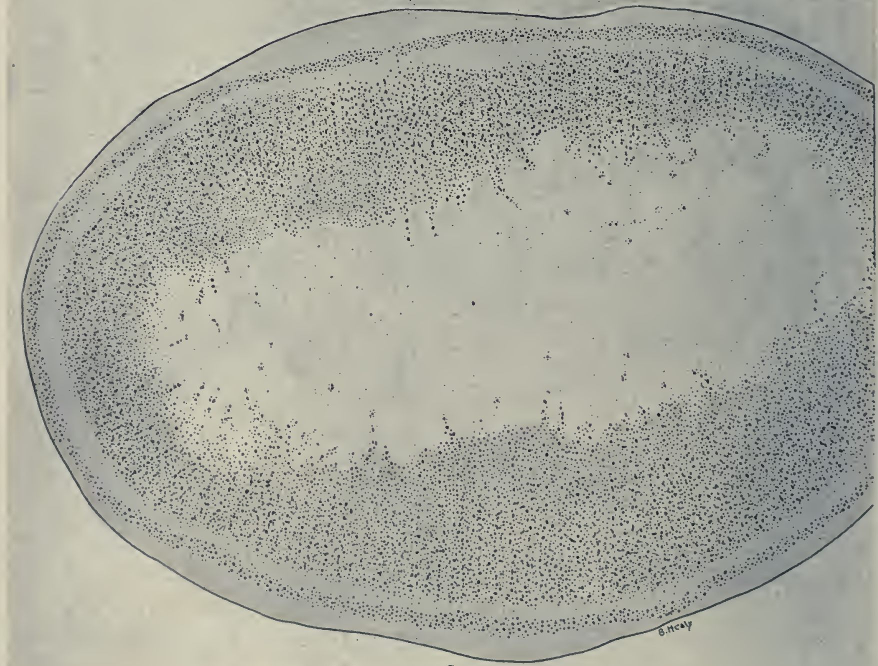
In distribution the liposomes, as noted in the fresh sections, are nearly uniformly present throughout the cortex, although relatively few occur in the narrow clear band between the outer and middle zones. In size they vary from extremely fine to coarse. The largest, however, are much smaller than later, now rarely reaching half the nuclear diameter. They form in each cell a circumnuclear zone which appears very distinct in the scarlet red or osmic-stained sections. The liposomes in the cortical cell strands through the medulla of the newborn rat usually appear similar in size and number to those in the cortical cells elsewhere. No liposomes are present in the cells of the medulla proper, which appear as clear and unstained masses, intermingled with the cortical strands.

In a rat of the second day (F. 10.2), the cortical liposomes in frozen sections stained with scarlet red appear in general as in the new-born, possibly slightly more abundant in the middle zone. The cortical cell strands in the medulla, however, are apparently undergoing absorption, and their liposomes appear fewer and finer than elsewhere in the cortical cells. At three days (fig. 2), the cortical cell strands throughout the medulla are very inconspicuous, though still distinguishable under high power in many places by their content of very fine (rarely coarser) liposomes. In some places the corticomedullary border is now clearly defined, though in other places still irregular. The exact date at which the cortical cell strands in the medulla are absorbed, leaving a clean-cut corticomedullary boundary, is subject to individual variation.

Second week. In rats one week of age, in sections of the suprarenal stained with hematoxylin-eosin, under low magnification, the cortical zones appear as in the new-born, but the medulla is much lighter in appearance and now appears separated from the cortex by a sharply defined border.



1



2

Under higher magnification the cortical zone cells appear in general similar to those of the new-born. The inner-zone cells, however, no longer intermingle with those of the medulla, but (aside from a few scattered islands of cortical cells) present a fairly even and very distinct line of demarcation. Next to this border, the cortical cells frequently appear flattened and more or less atrophic, probably due chiefly to absorption by the expanding medulla.

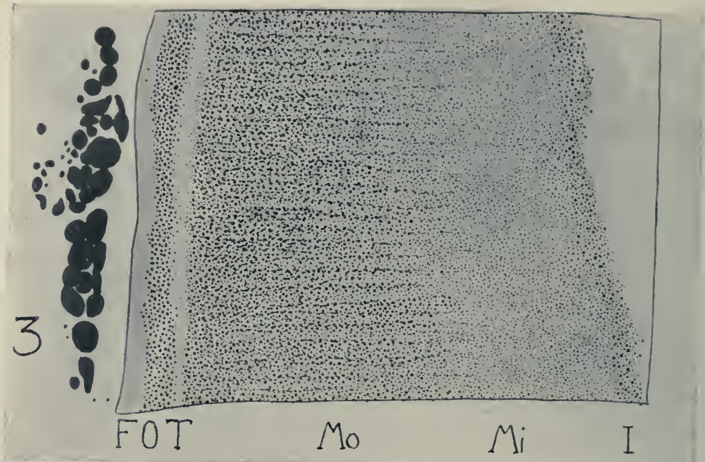
The medulla has become confluent as a central mass, which may also reach the surface at the hilus (compare fig. 10). It has undergone a marked change in structure and appearance. The stroma is well differentiated, forming an irregular syncytial net work containing elongated nuclei and fine fibrillae. The parenchyma cells are likewise syncytial. The cytoplasm is more abundant and presents small vacuolated (non-lipoidal) spaces, variable in size and number. The fine violet cytoplasmic granules are present, but variable and usually scanty. The nuclei are somewhat variable in form, but usually spheroidal and vesicular, very slightly chromatic. Some are smaller and more deeply staining, occasionally even pycnotic. Bilobed dumb-bell-shaped nuclei are occasionally seen in the medulla (as also in the cortex), but these are comparatively rare. It is doubtful whether they are to be considered as evidence of amitotic cell division. The deeply staining masses (sympatho-chromaffin cells) mentioned in the new-born occur rarely in the suprarenal medulla at one week.

In a rat at eight days (F 11.3) frozen sections unstained or stained with scarlet red or osmic acid show the liposomes some-

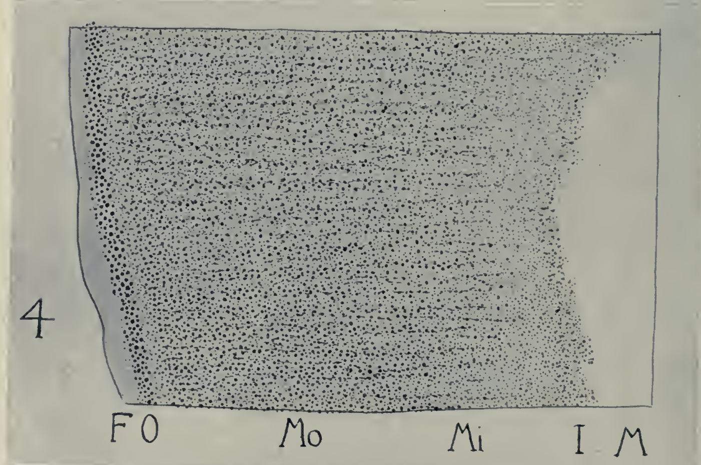
Fig. 1 Longitudinal section of the suprarenal gland of a new-born albino rat (F 8.1). Formalin fixation; frozen section stained with Herxheimer's scarlet red. Liposomes visible in the cortex and in the cortical strands throughout the medulla. The light band, relatively lipoid-free, which separates the outer and middle cortical zones is already evident. × 80.

Fig. 2 Longitudinal section of the suprarenal gland in an albino rat three days old (F 11.1). Formalin fixation; frozen section stained with Herxheimer's scarlet red. The medulla is confluent, and the intermixed cortical strands are undergoing absorption, with the establishment of a definite corticomedullary boundary. × 80.

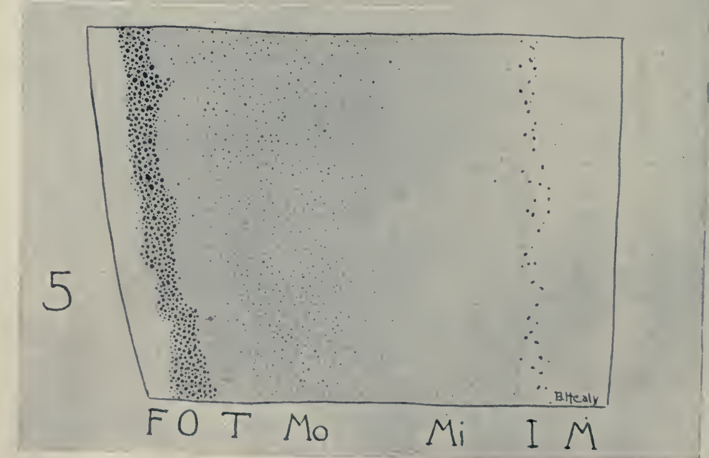
3



4



5



what similar to those of the first week. Those of the outer zone are relatively most abundant, varying from fine to coarse, the largest approaching nuclear size. The subjacent narrow light band is relatively free from lipoids, with only a few fine granules. In the middle zone the liposomes are less uniformly distributed than heretofore, becoming more abundant in the outer half of the zone. They are less abundant in the inner zone. Here occur a few coarsely granular cells, which also appear as individual cells or small groups sparsely scattered through the medulla. These islands represent unabsorbed remnants of the earlier cortical strands.

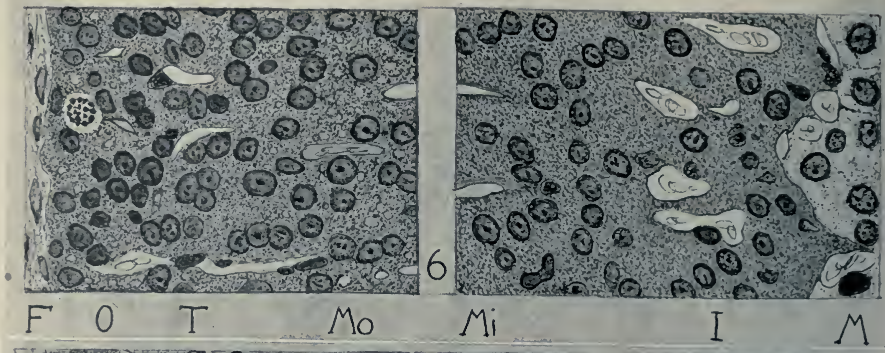
The chromaffin reaction of the medulla, though distinct, is still comparatively weak, as during the first week.

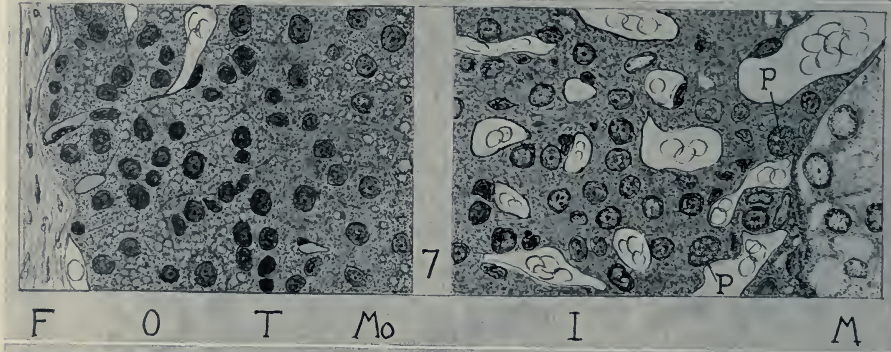
At ten to fourteen days, the suprarenal in general structure and appearance, in sections stained with hematoxylin-eosin, is similar to that at one week. Lipoidal vacuoles are becoming more abundant in the outer and especially the middle cortical zone. The reddish (eosinophile) cytoplasmic granules of the cortical cells are in contrast with the pale violet (faintly basophile) granules of the medulla cells. The cortical cells of the inner zone are usually normal in appearance, but frequently atrophic and degenerated, especially in certain areas. Large sympathetic ganglion cells appear in the medulla.

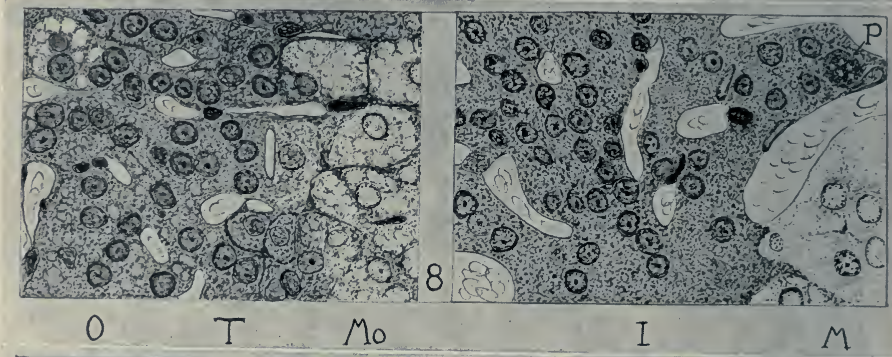
Fig. 3 Portion of a section of the suprarenal gland in a normal adult albino rat (F 3.1). Formalin fixation; frozen section stained with Herxheimer's scarlet red. Liposomes most abundant in the outer cortical zone and the outer half of the middle zone. *F*, fibrous capsule, with ordinary fat droplets in the tissue outside; *O*, outer zone (glomerulosa); *T*, transition band, relatively lipoid-free; *Mo*, outer part of middle zone (fasciculata); *Mi*, inner part of middle zone; *I*, inner zone (reticularis); *M*, medulla. × 90.

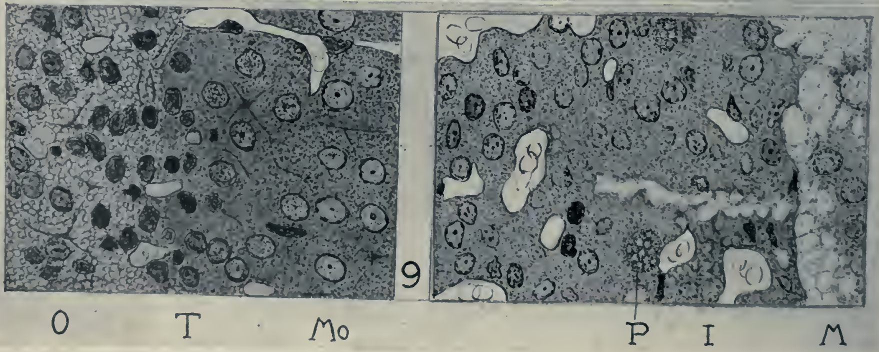
Fig. 4 Portion of a section of the suprarenal gland of an adult albino rat (F 3.2), after nine days of acute inanition. Formalin fixation; frozen section stained with Herxheimer's scarlet red. For explanations, see figure 3. No apparent decrease in the liposomes, which appear more uniformly distributed throughout the cortex. The light band at the transition between outer and middle zones is obscured. × 90.

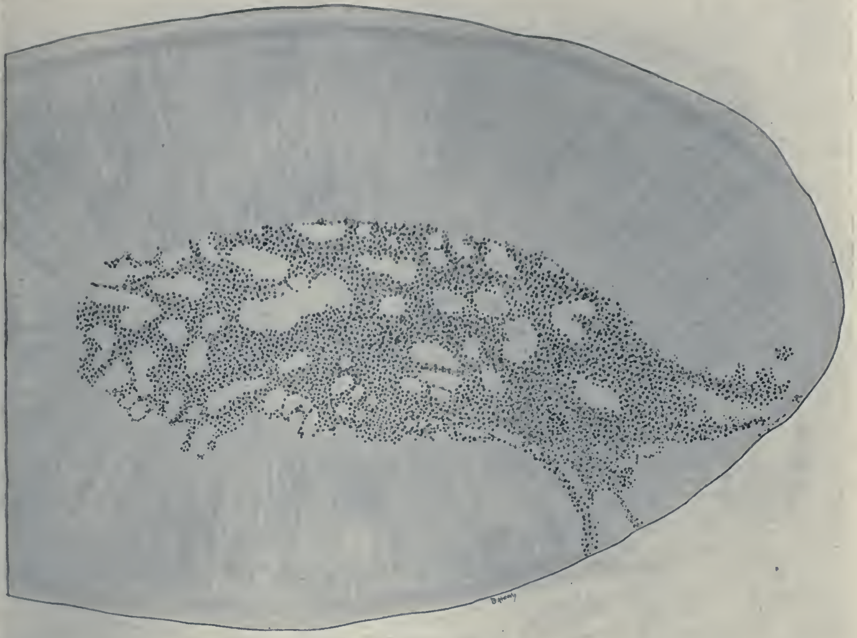
Fig. 5 Portion of a section of the suprarenal gland of an adult albino rat (F 4.2) after twelve days of acute inanition. Formalin fixation; frozen section stained with Herxheimer's scarlet red. The liposomes persist in the outer zone (*O*), but have nearly all disappeared elsewhere. A few are barely visible in the outer half of the middle zone (*Mo*), and in the inner zone (*I*). × 90.


6


7


8


9



10

Fig. 6 Portion of a section of the suprarenal gland of a normal albino rat (J 1.1) three weeks old. Zenker fixation, paraffin section stained with hematoxylin-eosin. Most of the middle cortical zone is omitted. *F*, fibrous capsule; *O*, outer zone (glomerulosa), one cell in mitosis; *T*, transition band, nearly lipoid-free; *Mo*, outer part, and *Mi*, inner part of middle zone (fasciculata); *I*, inner zone (reticularis); *M*, medulla. × 300.

Fig. 7 Portion of a section of the suprarenal gland of an albino rat (S 7.31) held at constant body weight by underfeeding from three to ten weeks of age. For explanations, see figure 6. Progressive differentiation of lipoidal vacuoles in the outer zone (*O*) and of pigment (*P*) in the inner zone (*I*). Some cell atrophy, with hyperemia and increased degeneration in the inner zone. × 300.

Fig. 8 Portion of a section of the suprarenal gland of a normal albino rat (St 7.45) ten weeks of age. For explanations, see figure 6. This represents the normal adult structure. Lipoidal vacuoles well marked in the outer zone (*O*) and outer part of the middle zone (*Mo*), but rare in the transition band (*T*). One pigment mass (*P*) visible. × 300.

Fig. 9 Portion of a section of the suprarenal gland of an adult albino rat (J 1.5) subjected to chronic inanition for five weeks. For explanations, see figure 6. The lipoidal vacuoles have disappeared, except in the outer zone (*O*). Marked atrophy with nuclear and cytoplasmic degeneration in the inner cortical zone (*I*). Three pigment masses (*P*) visible. × 300.

Fig. 10 Longitudinal section of the suprarenal gland in an adult albino rat (F 9.3) after ten days of acute inanition. Fixation in Müller's fluid; frozen section (unstained). Normal chromaffin reaction in the medulla, which in this case extends to the surface at the hilus. × 80.

In a rat at seventeen days (F 7.1) the fresh frozen sections of the formalin-hardened gland show the characteristic cortical opacity due to the liposomes. The clear line between the outer and middle zones is distinct. The opacity decreases greatly in the inner half of the middle zone and in the inner zone, the medulla being perfectly clear.

This irregular distribution of the liposomes is still more apparent when the sections are stained with scarlet red or osmic acid. The liposomes are most abundant in the outer zone and the outer half of the middle zone, where the largest droplets now approach nuclear size. Toward the inner cortical zone they become fewer and finer. Some of the inner zone cells are entirely free from liposomes, others occasionally appear well filled. Aside from a few scattered cortical cell islands, no liposomes appear in the medulla.

The other supararenal gland from this rat was hardened in Müller's fluid. The chromaffin reaction of the medulla is more definite than in the earlier stages, though not so intense as later. All of the medulla parenchyma cells are stained light brown, the nucleus darker than the cytoplasm. The vacuoles of various size in the medulla cells are unstained, not giving the chromaffin reaction. The blood in the vascular spaces of the medulla occasionally presents a reddish-brown color to a variable extent (probably from absorbed epinephrin).

At three weeks. The paraffin sections were stained as usual. The outer cortical zone (fig. 6, *O*) is narrow, usually 6 to 8 (rarely 12) cells deep. The cells are in irregular masses or columns, separated by blood capillaries. Cell boundaries are ill defined. The cytoplasm is scanty, contains fine eosinophile granules and more numerous lipoidal vacuoles. The nuclei are hyperchromatic, some almost pycnotic. The deepest cells (fig. 6, *T*) are transitional to the middle zone, and form a narrow zone nearly free from lipoidal vacuoles.

The middle cortical zone is broadest. The cells (fig. 6, *Mo*, *Mi*) are arranged in very distinct cell columns, radially arranged, and usually but one cell wide. The radial cell columns are separated by blood capillaries, which become wider toward the center of the gland. Cell boundaries are more distinct than heretofore. The relatively abundant cyoplasm is filled with characteristic eosinophile granules and a variable number of lipoidal vacuoles. In some cases these vacuoles appear no more numerous than in the second week. In others they are more abundant especially in the outer half of the middle zone, whereby these cells become much larger than the cells of the inner half of the middle zone. The nuclei are typical in structure, spherical, and moderately rich in chromatin. Atrophic cells occasionally occur in various stages of degeneration.

The inner zone (zona reticularis, fig. 6, *I*) is rather narrow, but of variable width, representing the area of irregular cell columns next to the medulla. The irregularity of structure is probably associated with the process of absorption accompanying the expansion of the medulla, although only occasionally are the border cells flattened as though atrophic from pressure. Most of the cells in the inner zone are similar to those of the adjacent middle zone, the cytoplasm containing eosinophile granules and a few small lipoidal vacuoles. Some scattered cells show various stages of degeneration and disintegration. Occasional islets of such cortial cells occur also in the adjacent medulla, but rarely deeper, toward the center of the medulla, as in the earlier stages.

The medulla in stained sections (fig. 6, *M*) appears very light, in strong contrast with the darker cortex. (The converse is true if the suprarenal gland has been fixed in Zenker-formol instead of Zenker's fluid, the sections being stained with hematoxylin.) The parenchyma forms irregular cell masses, separated by delicate fibrous stroma (with elongated nuclei) enclosing wide, sinusoidal blood-vessels. The cytoplasm of the parenchyma cells is abundant, containing pale violet (chromaffin?) granules and numerous non-lipoidal vacuoles, variable in size and number. The nuclei are typically vesicular in form and only moderately chromatic. A few are smaller and more deeply staining, sometimes pycnotic. Degenerative cells with karyolytic nuclei are rarely seen. Occasional large spherical sympathetic ganglion cells and bundles of non-medullated fibers appear.

At eight weeks. The appearance of hematoxylin-stained sections under low power is similar to that at three weeks. The cell structure in the outer and middle zones is also similar, with some increase in the size and number of lipoidal vacuoles, especially in the outer half of the middle zone. The inner zone shows in general a more atrophic appearance, and occasional pigment cells occur for the first time. These are parenchyma cells containing one (sometimes two) irregularly spheroidal, light yellowish-brown, coarsely granular masses. As will be shown later, this pigment is probably a lipochrome. The nuclei of these pigment cells are irregular, sometimes central, sometimes crowded to the side of the cell and flattened. The inner zone cells bordering on the medulla are usually more or less flattened and atrophic in appearance. The medulla appears similar to that at three weeks.

At ten weeks to adult. At ten weeks, the histological structure of the suprarenal (fig. 8) has reached practically the adult condition. In fact, although the gland increases in size, but few changes in structure are noticeable after the eight weeks' stage previously described. In the usual hematoxylin-stained sections the structure appears as follows (fig. 8):

The outer cortical zone (fig. 8. *O*) remains narrow, 6 to 12 cells deep. It is somewhat variable in structure, with irregular cell cords separated by blood capillaries. The cells remain relatively small, with nuclei of variable form and structure. The cytoplasm is relatively scanty and somewhat granular. It contains a variable amount of lipoidal vacuoles, usually giving the cytoplasm a honey-comb appearance. The deepest cells, on the border between the outer and middle zones (fig. 8, *T*), are relatively free from lipoidal vacuoles as found in the earlier stages.

In the middle zone the only noteworthy change is in the amount of lipoids, as shown by the vacuoles. These increase, but to a variable extent. They are larger and more numerous in the outer half of the zone (fig. 8, *Mo*), but smaller and fewer in the inner half. In the larger cells of the outer part, the cytoplasm presents a reticulated (honey-comb) appearance, like that of sebaceous gland cells. In cells with fewer lipoidal vacuoles, the eosinophile granules are more conspicuous. Occasionally basophilic granules also occur. These are especially evident in preparations stained with iron-hematoxylin, and probably in part correspond to the 'corps sidérophiles' or the mitochondria described by various French investigators. The nuclei are usually central in position, spherical, and typical in structure. A few degenerative cells occur as heretofore.

The inner zone (fig. 8, *I*) appears in general similar to that at eight weeks, the outer cells of the zone being transitional to the middle zone and the inner cells (next to the medulla) being typically more or less atrophic in structure. Atrophic or degenerative changes have likewise been described in the inner cortical zone of various animals (including the rat) by Gottschau ('83), Pfaundler ('92) Soulié ('03) and others. According to Kolmer ('12 a, '12 b) these degenerative changes are increased by pregnancy (guinea-pig).

Pigment cells in the inner cortical zone of the rat at ten weeks occur more frequently than at eight weeks, but in variable degree. They are somewhat variable in number, often numerous, especially in the older rats, and may even extend somewhat into the middle zone. The nuclei of the pigment cells may be central in position, surrounded by the light yellowish-brown or greenish yellow granular masses. In this case the nucleus is irregular in form, but it is often pushed aside and flattened. The pigment is still visible, though inconspicuous, in stained paraffin sections. In thin sections the pigmented substance frequently appears vacuolated (fig. 8, *P*). In fresh frozen sections the pigment cells are clearly visible, and they are deeply stained by osmic acid or scarlet red. This would indicate that the pigment is a lipochrome, probably derived by a transformation of lipoids or other fatty substances. The vacuoles probably represent untransformed lipoids, observed by Ewald ('02) and Da Costa ('13).

The cortical fats (lipoids) in general are best studied in frozen sections of suprarenals, either fresh or hardened a few hours in formalin. In the unstained sections the distribution of the liposomes is clearly evident on account of the varying degrees of opacity caused by them. The extracapsular fat droplets, on the other hand, appear clear and light. The liposomes present the same reactions mentioned for the new-born.

The amount and distribution of the liposomes are most clearly shown in the frozen sections stained with scarlet red (fig. 4). In the narrow outer zone, they are usually very abundant and closely packed, the largest droplets somewhat exceeding the average nuclear size. The subjacent clear line or narrow band is relatively (but not absolutely) free from liposomes. They are most abundant and closely packed in the outer half of the middle zone, where the largest may reach double the average nuclear diameter. In the inner half of the middle zone they become more or less reduced in amount, and are usually scanty in the inner cortical zone (aside from those associated with the pigment cells). No liposomes are present in the medulla, aside from occasional groups which appear in the cortical islands near the boundary.

The distribution of liposomes in sections stained with osmic acid corresponds closely with that in sections stained with scarlet red. The striking contrast in the staining reactions of the cortical lipoids and the extracapsular ordinary fat was mentioned in the new-born suprarenal, and is constant. The pigment cells (lipochrome) of the inner cortical zone frequently react more like the ordinary fat, however, staining a lighter reddish than the liposomes with the scarlet red and darker than the liposomes in preparations stained with osmic acid.

The amount and distribution of lipoids, as is well known, vary much according to age and species. In general, the formation of lipoids begins during the prenatal period (Poll, '05; Starkel and Wegrzynowski, '10), and increases during postnatal life, though a decrease in the human suprarenal fat after childhood is noted by Napp ('05). As to species the lipoidal content appears greatest in man, carnivora, and rodents, less in ruminants and pachyderms (Ciaccio, '10, and others). The lipoids are usually, as in the rat, most abundant in the middle zone, with less in the outer and inner zones. In some cases, however, a greater abundance of lipoids of the outer zone has been observed (Hultgren and Anderson, '99; Ewald '02; Napp, '05; Bonnamour, '05 b; Starkel and

Wegrzynowski,'10); and in the sheep, according to Mayer, André, Mulon and Schaeffer ('12), the sparse lipoidal granules are located exclusively in the outer zone. The characteristic fat-free band between the zona glomerulosa and the fasciculata was noted in the rat by Bonnamour ('05 b). Babes and Jonesco ('08) likewise noted a scarcity in fat between these zones in the dog, but Mulon ('12), on the contrary, finds larger droplets at this level. The composition of the suprarenal liposomes is generally considered to be chiefly lecithin or cholesterin with a variable admixture of ordinary fats (compare Ciaccio, '10, and Kawamura, '11).

Pigment has often been described in the inner cortical zone of the guinea-pig (even in the albino, according to Mulon) and man, but less frequently in other forms. It was noted by Gottschau ('83) and Pfaundler ('92) in most mammals, Baroncini and Beretta ('01) in bats, and by Elliott and Tuckett ('06) sparsely in the duckbill and pigeon. Bonnamour ('05 a) found it variable in the dog and horse, and absent in the cat, rabbit, and marmot. He found it rarely in the rat, with no difference in pigment between white and black rats. DaCosta ('13) also noted a few pigment cells in the zona reticularis of Mus decumanus. Dewitzky ('12) likewise noted brownish pigment in the cortex of the rat at seven weeks. Delamere ('03) and others have noted that the amount of pigment usually increases with age, being rare or absent in the young. Mulon ('02, '03 a), Ciaccio ('05), Kolmer ('12 b), and others believe that the pigment formation may represent a secretion with physiological significance, and an increase during pregnancy (guinea-pig) is claimed by Kolmer ('12 a). As to its composition, this pigment has usually been considered as related to fat (lipochrome), although Starkel and Wegrzynowski ('10) and Thomas ('11) consider that the pigment appearing in the degenerating inner cortical zone of the suprarenal in the human new-born is of hemal origin and different from the pigment in the adult gland.

The suprarenal medulla in the rat at ten weeks and older may extend to the surface at the hilus (fig. 10), as found by Poll ('99). It is similar in structure to that described in earlier stages.

The stroma (also that in the inner cortical zone) stains indistinctly bluish with Mallory's anilin-blue connective-tissue stain. The parenchyma cells (fig. 8, *M*) are large. With Zenker's fixation and hematoxylin-eosin stain, the cytoplasm contains the usual faintly basophilic (chromaffin?) granules and occasionally a few spherical eosinophile bodies of variable size. The cell periphery frequently contains irregular vacuoles, non-lipoidal and of unknown significance. They are variable in size and are somewhat more prominent than in earlier stages. According to Ciaccio ('05), they are acidophile in reaction, like true nucleoli, and are comparable to 'plasmosomes.' The structures described by Ciaccio probably correspond to the eosinophile bodies referred to above, and not to the characteristic vacuoles. The nuclei, as heretofore, are typically vesicular, though a few of them are small and hyperchromatic (sometimes pycnotic). The blood sinuses, sympathetic ganglion cells, and occasional atrophic cortical islands occur as heretofore.

In preparations fixed in Müller's fluid, the chromaffin reaction of the medulla is always well marked (as in fig. 10). The parenchyma cells appear as brownish masses separated by the unstained vascular areas. There is some variation in the intensity of the reaction in different individuals, although the variation is not great in sections of the same thickness. It also usually appears fairly uniform throughout the medulla, all of the parenchyma cells being somewhat similar in their reaction. Some masses or clumps of cells may stain more deeply, however. The brownish color appears in the cytoplasm, which may appear homogeneous or granular (the granules being most distinct in very thin sections). The cytoplasmic vacuoles of various size remain perfectly clear and unstained. Whatever their nature, they evidently do not contain epinephrin. The nucleus gives the chromaffin reaction, being stained slightly darker than the cytoplasm. This was noted by Dostoiewsky ('86) confirming Henle (versus v. Brunn). According to Ciaccio ('05), Diamare claims that the chromaffin substance fills the whole cell, while Grynfeld, localizes it in the cytoplasmic granules. The reaction is weakened or lost a few hours post mortem (Dostoiewsky, '86; Ciaccio.

'05, and others), and is also affected by anesthetics (Schur and Wiesel, '08; Hornowski, '09). The chromaffin reaction sometimes appears also to a variable extent within the blood-vessels of the medulla of the rat, as has often been noted in other animals by various observers.

2. Changes in young rats stunted by underfeeding

Underfed from birth. In a rat (St 80.9) underfed from birth to twelve days and reaching a body weight of 8.9 grams, the suprarenal gland has increased in weight to 0.0026 gram, which is about normal for seven days of age. The normal differentiation of the suprarenal cortex and medulla has occurred, and the structure (in sections stained with hematoxylin) is essentially similar to that of the normal rat at seven days. Thus the histological differentiation has continued, as in a normal gland of corresponding weight, although(as previously shown) the rate of mitosis has been greatly diminished.

In a rat (St 247.5) underfed from birth to seven days, there is apparently no increase in the weight of the suprarenal. Frozen sections stained with scarlet red and osmic acid show the cortical liposomes somewhat similar in appearance to those in the newborn, but progressive absorption in the cortical cell strands of the medulla is evident. The chromaffin reaction is normal.

In another rat (St 228.4) underfed from birth to fifty-eight days, the suprarenal gland has increased markedly in weight and shows a progressive differentiation of liposomes similar to those in a normal gland of similar weight (F 7.1). The chromaffin reaction in the medulla is variable. Some cell masses show a very definite reaction. In others it is entirely absent, giving the medulla a spotted appearance. This irregularity of the chromaffin reaction is probably due to the inanition, as it is not observed in the normal animals.

Rats at maintenance from three weeks to eight, ten, or twelve weeks of age. In these underfed young rats the body weight and suprarenal weight (table 1) are but little above the initial weight at three weeks. In general, the stained sections (Zenker's fixation, hematoxylin-eosin stain) of the suprarenal appear similar to those normal at three weeks, though certain changes are clearly evident (fig. 7).

The vacuoles in the outer-zone cells usually indicate a progressive lipoidal differentiation (fig. 7, *O*). While in a few cases the lipoidal vacuoles appear scanty (as normally at three weeks), in others the outer lipoid zone was as distinct and clearly differentiated as normally at ten weeks. In most cases the lipoidal content appears somewhere between these two extremes. The eosinophile cytoplasmic granules are somewhat indistinct.

The lipoidal vacuoles of the middle zone were also found increased in size and number in most cases, though more variably and not so definitely as in the outer zone. As in normal differentiation, the lipoids usually accumulate to a greater extent in the outer half of the middle zone. Otherwise there is no constant change to be noted, excepting apparently a somewhat greater number of degenerating cells than occurs normally.

The inner zone (fig. 7, *I*), however, when compared with the normal shows the most conspicuous changes. This zone is constantly hyperemic and very markedly atrophic. The cells in general appear greatly decreased in size, and many show various stages of nuclear and cytoplasmic degeneration. More cells are flattened at the medullary border than occurs normally. The nuclei are frequently pycnotic or karyolytic. Pigment masses (not present at three weeks) appear (fig. 7, *P*) and are even more abundant than in the normal gland at ten weeks.

The medulla (fig. 7, *M*) shows relatively less change than any other part of the suprarenal. In most cases it resembles closely the normal at three weeks, though in some cases there is an evident increase in the proportion of smaller, hyperchromatic (occasionally pycnotic or karyolytic) nuclei. The cytoplasmic granules are usually indistinct. No chromaffin tests were made in these cases.

Maintenance from three to fifteen or twenty weeks of age. In these two young rats (St 33.1 and St 38.8) held at maintenance for unusually long periods, the changes in the suprarenal are more pronounced. The glands were fixed in Zenker's fluid, sectioned and stained as usual. In general, the nuclei appear more hyperchromatic, though not much more than in the normal gland at three weeks. Although the fat in the fibrous capsule around the gland has apparently almost entirely disappeared, the lipoidal vacuoles of the outer zone of the cortex are abundant. In the middle zone, however, they are apparently not more numerous than in the normal at three weeks. In the inner part of the middle zone many cells are degenerating some in various stages of disintegration. The atrophic degeneration and pigment formation in the inner cortical zone is very marked. The greenish-yellow, vacuolated pigment masses in many cases have entirely filled the cells and are quite numerous. The medulla cells show increased vacuolization, sparser granulation, and hyperchromatic (frequently pycnotic, some karyolytic) nuclei. In general, however, the structure is less changed than in the cortex.

3. Changes in young rats refed after stunting by underfeeding

In the rats refed fully after maintenance from three to twelve weeks of age, the suprarenal gland rapidly returns to approximately normal structure. Even after one week of refeeding the cortex appears distinctly lighter in stained sections, due to a decrease in the chromatin content of the nuclei. The lipoids accumulate more abundantly (especially in the outer half of the middle zone) and the inner cortical zone becomes somewhat less atrophic in appearance. By the end of two weeks of refeeding, the gland has reached nearly normal structure, although some areas of degeneration may persist for longer periods. Even those held at maintenance up to twenty weeks of age (S 33.118 and S 33.120), with permanently stunted body weight, show practically normal structure in the suprarenals after being refed fully up to about one year of age.

4. Changes in adult rats after acute or chronic inanition

Adult acute inanition. Adult rats were given water only for seven to twelve days, with loss in body weight of 29 to 45 per cent (table 1 D). The suprarenal glands, as previously shown

(Jackson, '15 a), lose but little in absolute weight during adult inanition. The cells (especially those of the middle cortical zone and the medulla) during acute inanition apparently diminish in size, however, as shown in table 4. The decrease in cell size is counterbalanced by hyperemia of the cortex, though apparently the reverse (decrease in proportion of vascular stroma) occurs in the medulla.

Sections of the suprarenal, stained with hematoxylin-eosin, show changes in histological structure. The cytoplasm is reduced in amount and the eosinophile granules usually indistinct. The nuclei are variable, frequently hypochromatic. More frequently, however, they appear hyperchromatic, though less so in the outer half of the middle zone. Pycnosis and deformity of the nuclei are frequent, as described by Bonnamour ('05 b) in the outer cortical zone of the starved rat. The lipoidal vacuoles are variable. Usually, however, they are absent or considerably reduced in number and size in the middle zone, though persisting nearly unchanged in the outer zone. The eosinophile granules of the cortical cells, though sometimes indistinct, are often well preserved and become more evident with the reduction in the amount of lipoids present.

The inner cortical zone shows the hyperemia and atrophic condition usually more pronounced than in the normal animal. Pigment masses are frequent, but it is somewhat doubtful whether they are increased in number. Degenerating cells with pycnotic or karyolytic nuclei in various stages of disintegration are numerous.

Scarlet red or osmic staining of fresh frozen sections reveals the cortical liposomes much more abundant than would be suspected from the ordinary stained paraffin sections. In some cases the usual fat-free boundary line between the outer and middle zones is nearly obliterated by an increased development of liposomes, and they may appear more uniformly scattered through the middle zone, and even the inner zone (fig. 4)..

This tendency toward a more uniform distribution of the liposomes throughout the cortex was noted in three of the eight cases of adult acute inanition stained especially for lipoids, though the total amount of the lipoids appears somewhat reduced in one of them (F 1.2). A similar 'Verbreitung der lipoidhaltigen Schichten' was observed by Landau ('13 b) in fasting guinea-pigs.

In five of the eight rats in my series, however, the change consists in a very marked reduction in the liposomes of the middle and inner zones. Under the low power of the microscope only a few liposomes may remain visible in the outer half of the middle zone, the remainder of the middle and inner zones being apparently free from them (fig. 5). Under higher power, however, numerous extremely fine granules are often still visible in all these cortical cells. The effect in such cases is therefore apparently merely to reduce the liposomes very much in size. In other cases, the liposomes have entirely disappeared. The pigment cells near the corticomedullary zone apparently persist nearly unchanged, contrary to Rondoni and Montagnani ('15), who found a decrease in fasting guinea-pigs.

In striking contrast with the remainder of the cortex is the narrow outer zone, in which the liposomes persist with great tenacity (fig. 5). With the scarlet stain, this zone therefore appears nearly unchanged as a deep reddish band, while the remainder of the cortex appears pale, nearly colorless, or with a pale, diffuse reddish color suggesting some fat-like substance in solution. Osmic preparations give corresponding pictures. In only one case (F 9.3) the outer zone in places appears somewhat broken and irregular. The tenacity of the outer lipoidal zone is not due to any visible difference in the structure or composition of the liposomes in this zone. Landau ('13 b), however, finds that in the fasting guinea-pig, although there is no decrease in the total lipoid content, the cholesterin decreases in all but the outer cortical zone. It may be dependent upon the vascular arrangement, as all of the blood to the cortex passes first through the capillaries of this zone (Flint, '00).

The amount of decrease in the liposomes of the suprarenal cortex is usually, but not always, somewhat proportional to the length of the fasting period or to the loss in body weight. In general, the lipoids appear much more resistant to inanition than does the ordinary fat just outside the suprarenal capsule.

The suprarenal medulla is often considerably affected during acute inanition, though usually less so than is the cortex. The cytoplasm frequently becomes more vacuolated, although the characteristic (chromaffin?) granules persist in the parenchyma cells. The nuclei may become more hyperchromatic, and a larger proportion are pycnotic. In many cases the nuclei are hypochromatic, undergoing chromatolysis. Some areas of marked cellular degeneration occur.

The chromaffin reaction is apparently but slightly (if at all) affected by the inanition experiments. The slight variations in the intensity of the brown color are apparently no greater than appear in the normal controls. Even in the rat starved twelve days with loss of 45 per cent in body weight (F 5.2) the chromaffin reaction of the medulla appears fully as intense as in any of the controls. This rat was still active when killed, but the reaction persists in another (F 9.3) which was killed while very weak and near death with a loss of 33 per cent in body weight after ten days of acute inanition (fig. 10).

In only one case (F 6.2) is there a marked decrease, only traces of the chromaffin reaction being present. This was probably a postmortem change, as the rat was found dead. It had lost only 34 per cent in body weight after seven days of inanition. It therefore appears that in adult rats acute inanition produces no appreciable decrease in the chromaffin reaction of the suprarenal medulla, even (aside from postmortem changes) in those starved to death.

Adult chronic inanition. In six adult rats underfed thirty to thirty-five days with gradual loss in body weight amounting to 33 to 38 per cent, the histological changes (in the ordinary preparations) were found in general very similar to those after acute inanition (fig. 9). These include a general atrophy of cells and nuclei with a variable reduction in the amount of lipoids in the middle (but not in the outer) zone (fig. 9, O). There is similarly a pronounced cellular atrophy in the inner zone, and in one case (J 1.5, fig. 9, *I*) the vacuolated cytoplasm in a few places had apparently disintegrated to form extensive intercellular spaces, similar to the condition described in the human suprarenal by Meyer ('17).

The cells usually undergo simple atrophy, however. Many of the nuclei appear chromatolytic, some pycnotic. There is apparently no change in the number and appearance of the pigment cells, as a rule, although sometimes they appear increased in number (fig. 9, *P*). The changes in the medulla are similar to those noted under acute inanition. The special lipoid and chromaffin stains were not employed in the chronic-inanition series.

From the foregoing it appears that during inanition in the albino rat the behavior of the suprarenal lipoids is somewhat variable according to circumstances. In young rats severely stunted by underfeeding there is no apparent loss, the liposomes continuing to differentiate as in the normal gland of corresponding weight. Likewise in older rats there is sometimes no evident decrease, but instead a tendency to more uniform distribution of the liposomes throughout the cortex. This appears characteristic where the inanition has not been carried to extremes. No decrease in suprarenal fat (lipoids) during inanition has also been observed by Frederici ('03) in the guinea-pig, Traina ('04) in man and rabbit, Napp ('05) in man, Kawamura ('11) in man, Landau ('13 b) in man, cat and guinea-pig. Bonnamour ('05 b) even finds an increase in the starved rat, cat, rabbit, and guinea-pig. Ciaccio (according to Landau, '13 b) during inanition found first an increase, with subsequent decrease, in the suprarenal fat content.

In most cases of acute and chronic inanition in the rat, especially in those carried to extremes, there is a well-marked decrease in the suprarenal lipoids, although they are retained in the outer cortical zone with remarkable tenacity in all cases. A decrease in the fat (lipoids) of the suprarenal during inanition has been observed by Orth ('93) in man, Beneke in man and animals, Ewald ('02) in the rabbit, Herman ('05) in man, Ponomarew ('14) in the mouse, and Rondoni and Montagnani ('15) in the guinea-pig. In the latter two investigations, more or less retention of fat in the zona glomerulosa (of mice and guinea-pigs) is mentioned, which would be in agreement with my observations on the rat. A variable decrease in suprarenal fat (lipoids) as a result of various toxic conditions has been noted by various investigators in man and lower animals. The apparently contradictory results of different observers as to the effect of inanition upon the suprarenal fat (lipoids) is doubtless due to variations in the age, species, and individual animals, as well as to variations in the extent and character of the inanition.

In hibernating gophers, Mann ('16) found no change in the lipoid content of the suprarenal. Frederici ('03) found no decrease in hibernating bats, but a progressive decrease is noted by Baroncini and Beretta ('01) and Ciaccio ('10). Bonnamour ('05 b) and Ciaccio ('10) found a decrease in hibernating hedgehogs.

As to the effect of inanition upon the chromaffin reaction of the suprarenal medulla, my results for the rat are more uniform. They indicate that (with rare exceptions in extreme cases, especially in the younger stunted rats) there is no appreciable decrease in the reaction, except as a result of postmortem changes. This is in agreement with the results of Luksch ('05, '11) and Kuriyama ('18), who found no decrease in the epinephrin content of starved rabbits, but contrary to Venulet and Dmitrowsky ('10) in the rabbit, Borberg ('12) in the cat and guinea-pig, Rondoni and Montagnani ('15) in the guinea-pig, and Pellegrini ('16) in the later stages of fasting. In most cases, these positive results were obtained on animals starved to death, and are therefore probably explainable as due to postmortem changes. Effect of anesthetics and acid in the fixative (for example, in the formalin used for Wiesel's mixture) are also possible sources of error, as I have learned by experience.

MORPHOGENESIS OF THE SUPRARENAL GLAND

Some general features in the process of morphogenesis of the suprarenal gland may now be discussed. As is well known (for details in various species compare Poll, ('05), the medulla arises in the embryo in connection with the sympathetic system, and later migrates, usually in the form of multiple, small sympathochromaffin cords or masses, which (in mammals) pass through the cortical anlage and finally collect in the center of the gland. Here they later become confluent, a few cells ('sympathoblasts') forming the sympathetic ganglion cells, the majority 'phäochromoblasts' or 'chromaffinoblasts') ripening into the characteristic parenchyma of the medulla.

The time at which this confluence of the medulla is finally completed varies in different mammals, but is usually during the late fetal period. In Echidna, however, the immigration of the sympatho-chromaffin anlage is greatly delayed, and the suprarenal medulla is not formed until long after birth (Keibel, '04). Also in the new-born mouse (Inaba, '91), cat, dog, and guinea-pig (Soulié, '03) and occasionally even in the human new-born (Starkel and Wegrzynowski, '10; Zuckerlandl, '12), the process is still incomplete and admixture of cortex and medulla persists to a variable extent after birth. In the mouse, Inaba ('91) found the corticomedullary boundary line still indefinite at ten days, but distinct at thirty days.

Soulié ('03) described the confluence of the medulla in the suprarenal of the rat (Mus decumanus) as appearing in the 25-mm. fetus. Dewitzky ('12), however, found the suprarenal medulla in the rat very ill defined at birth, becoming distinct at three days and thereafter. This is in general agreement with my observations, although I find the confluence of the medulla in the albino rat to be a gradual process and subject to some individual variation. It is usually completed by the end of the first week. Even in the adult, however, short cords of cortical tissue may occasionally extend into the medulla, as observed by Bonnamour ('05 a) in the rat and rabbit, and small cortical islands occur near the margin of the medulla.

As to the exact manner in which the confluence occurs, but few definite statements appear in the literature. Flint ('00) mentions appearances of pressure atrophy in the cortical strands in the medulla of the suprarenal in the fetal pig, and Soulié ('03) states that the cells of the medulla anlage "etouffent peu à peu les cordons corticaux emprisonnés au stade de pénétration." It seems to be generally assumed, however, as is stated definitely by Inaba ('91) for the mouse, that these cortical cell strands and masses intermingled with the medulla are finally displaced and squeezed out of the medullary mass. In the rat, at least, there is no evidence that such a displacement occurs. It appears rather that the medulla becomes confluent through degeneration and absorption of the intermingled cortical remnants. This process is difficult to observe in preparations fixed and stained in the usual manner, on which account it is easily overlooked, but it is clearly evident in frozen sections stained with Herxheimer's scarlet red. In such preparations, the stained liposomes reveal the atrophic cortical cells undergoing gradual atrophy and absorption. A careful study of similar preparations would probably show that in other forms the primitive cortical strands in the medulla likewise undergo degeneration and absorption.

The process of absorption of the cortical tissue continues during the postnatal growth and development of the suprarenal gland, associated with the expansion of the medulla. The continued postnatal growth of the medulla has been noted in the rat, cat, rabbit, and guinea-pig by Elliott and Tuckett ('06) and in man by Scheel ('08), Starkel and Wegrzynowski ('10), Thomas ('11). Kern ('11), and others. The continued postnatal increase in the absolute volume of the medulla in the rat is confirmed by the extensive data in the present study.

This expansion of the medulla necessarily involves an encroachment upon the space formerly occupied by cortex. A priori, this might happen in three ways: 1. There might be a corresponding interstitial growth and expansion of the adjacent cortex. This, however, would require continued multiplication and growth of the cells in the inner cortical zone, and numerous observers (as previously shown) agree that postnatal cell division in the inner cortical zone during postnatal growth rarely or never occurs.

2. In the absence of cell division with interstitial growth, the inner cortical zone might remain passive and be mechanically displaced by the expansion of the medulla. Such a displacement, however, would inevitably result in a very marked flattening of the cortical cells on the adjacent surface of the expanding medulla It cannot be denied that occasionally such a flattening does appear, but it is irregular and inconstant. Indeed the characteristic

irregularity of arrangement of the cell cords in the zona reticularis is perhaps in part due to the pressure of the expanding medulla. In the rat, however, and apparently in other forms, the histological structure of the inner (reticular) zone at the corticomedullary border does not, in general, support the idea of a mechanical displacement by pressure.

3. The remaining possibility is that there is an actual absorption and removal of the cortex at the corticomedullary border. This theory is strongly supported by the available evidence. Hyperemia of the inner cortical zone, absence of cell division and a more or less well-marked cell atrophy with degeneration and pigment formation are (as has been shown) characteristic not only for the rat, but for mammals in general. In most cases, this process of absorption is comparatively slow and inconspicuous, as in the rat, but in the human infant it is more prominent. The extensive degenerative atrophy of the inner cortical zone of the suprarenal in the human new-born, as described by Starkel and Wegrzynowski ('10), Thomas ('11), Kern ('11), Elliott and Armour ('11), Landau ('13 a) and Lewis and Pappenheimer ('16), is therefore not a unique phenomenon, as heretofore supposed. It appears to be merely an exaggeration of the same fundamental process found in the development of the suprarenal in other mammals. It is thus incorrect to claim that nothing similar occurs in the lower animals (Kern, '11; Dewitzky, '12; Landau, '13 a). The erosion of the inner cortical zone is evidently a continuation of the same process of degeneration and absorption of the cortex which removes the cortical strands at the time of the original confluence of the medulla. Minot ('97), on the other hand, believed that the cells of the primitive medulla anlage disappear in the fetus, and agreed with the view of Gottschau ('83) that the permanent medulla is derived by transformation of the suprarenal cortex.

The process of cortical erosion by the medulla may be compared with that of the absorption of the cartilage by the osteogenic tissue in the zone of enchondral ossification. In both cases, small islands of the invaded tissue may persist for variable periods. It is significant that such cortical islands in the medulla of the suprarenal in animals after the earlier postnatal stages usually occur only in the immediate neighborhood of the corticomedullary border. Those originally in the central portion of the medulla have usually undergone complete absorption. Flint ('00), Pellegrino ('04), and others have described cortical islands in the medulla of the adult suprarenal, and they occur also in the rat; but these probably represent the results of later cortical erosion, rather than persistent embryonic remnants.

The exact nature of this absorptive process is uncertain. It does not appear to be a mere pressure atrophy, due to the expansion of the cortex, although this may be a factor of subordinate importance in the process. It would appear to be rather a chemical process of obscure nature, possibly a result of the contact between the essentially alien cortical and medullary tissues, not yet fully adapted to each other in their comparatively recent phylogenetic association. The observation by Lewis and Pappenheimer ('10) of similar involutional changes in accessory suprarenals composed of cortical tissue only would seem to invalidate this theory, but this point needs further investigation. It does not appear probable that the absorption of the cortex at the corticomedullary border is of functional significance, as claimed by Gottschau ('93) and Mulon ('02, '03 a, '03 b, '05 a, '05 b, '12).

If the inner zone of the cortex is subject to continued erosion and absorption during the morphogenesis of the suprarenal, it is evident that the zone must be constantly regenerated. As has been previously shown, the abundant evidence in various animals clearly establishes the fact that although during embryonic and fetal periods cell division occurs throughout the cortex, during postnatal development it becomes progressively restricted to the outer region of the cortex in general, and to the zona glomerulosa in particular. There is therefore during the postnatal growth period a continued renewal of the suprarenal cortex, the cytomorphosis of the cells including an origin in or near the outer zone (glomerulosa), a descent through the middle zone (fasciculata), and a final atrophy, absorption and removal in the inner zone (reticularis) at the corticomedullary border. The process may be compared with the cytomorphosis of the cells of the epidermis, where, however, the process is centroperipheral instead of peripherocentral in direction.

The weight or volume of the suprarenal gland as a whole, together with the relative volumes of the cortical and medullary constituents, will therefore vary according to the rate of expansion of the medulla, the rate of erosion of the cortex at the inner zone, and the rate of regeneration from the outer zone. Thus the postnatal involution of the inner zone in the human suprarenal is so rapid that it is not fully compensated by regeneration from the outer zone, or by expansion of the medulla; so the gland during the first year actually decreases in weight, as shown by the data of Scheel ('08) Starkel and Wegrzynowski ('10), and others. In the rat the retardarion in the growth of the gland during the first week is perhaps explainable upon the same basis. Subsequent changes in the absolute and relative volume of the cortex in the rat and other forms are evidently subject to much variation in different individuals and species. As a rule, however, as in the rat, the medulla appears relatively small in volume in the earlier prenatal stages (during immigration), expands rapidly to a maximum relative size following its confluence (early postnatal stages), and thereafter decreases relatively corresponding to the later more vigorous growth of the cortex (Canalis, '87; Hultgren and Anderson, '99; Soulié, '03; Elliott and Tuckett, '06; Scheel, '08; Starkel and Wegrzynowski, '10; Thomas, '11, and others).

SUMMARY

1. In the new-born rat, the suprarenal cortex and medulla are not yet distinctly separated, the cortical cell strands in the medulla being absorbed during the confluence of the medulla in the first week. During the second and third weeks after birth, the cortex increases from 75 or 80 per cent to about 90 per cent of the entire gland, by volume. It apparently continues to increase relatively to about 93 per cent at ten weeks of age, decreasing slightly in the adult. The medulla increases more slowly in absolute volume, thereby decreasing in relative volume

from 20 or 25 per cent of the gland at one week to 15 per cent at two weeks, 10 per cent at three weeks, and 7 per cent at ten weeks, increasing again slightly in the adult.

2. The relative volumes of cortex and medulla are subject to considerable individual variation. Their ratio shows no distinct difference according to sex and is not materially changed in young rats stunted by underfeeding or in adults subjected to acute or chronic inanition.

3. The vascular stroma (in comparison with parenchyma) normally shows considerable individual variabilty in relative volume, due chiefly to the varying distention of the blood-vessels. In general, however, there is evidently an increased vascularity upon passing from the outer zone of the cortex (average 13 per cent) toward the medulla (average 28 per cent), with no constant change according to age.

4. The changes in the volume of the vascular stroma during inanition are variable. In general, the stunted young rats show a marked hyperemia in the inner cortical zone, with relative anemia in the middle and outer ones; medulla unchanged. In stunted rats refed one to two weeks, the relative volume of the stroma in general returns toward normal, though the outer zone remains anemic. In adult rats with acute or chronic inanition the inner and middle cortical zones appear hyperemic, the medulla relatively anemic.

5. With the exception of the first week, there is in general an increase in the size of the suprarenal parenchyma cells from birth to ten weeks of age, with little or no increase thereafter. The average change in cell diameter for the various zones between birth and maturity is as follows: outer zone, 7 μ to 9 μ; middle zone (outer portion), 9 μ to 15 μ; middle zone (inner portion), 9 μ to 12 μ; inner zone remains about 9 μ; medulla, 8 μ to 16 μ.

6. The nuclei increase slightly in diameter during postnatal life, excepting those of the inner cortical zone. The cytoplasmic increase is much greater, however, so the nuclei in general lag behind in relative size (nucleus-plasma ratio). Thus the relative nuclear volume decreases, in the outer cortical zone, from about 44 per cent of the cell volume in the new-born to 23 per cent in

the adult; in the middle zone (outer part) from 33 per cent to 10 per cent; in the middle zone (inner part) from 28 per cent to 16 per cent; in the inner cortical zone from 28 per cent to 21 per cent; in the medulla from 46 per cent to 10 per cent.

7. In the underfed young rats stunted three to ten weeks or more, the suprarenal cells may increase in size (outer part of middle zone), or decrease (inner cortical zone and inner part of middle zone), or remain nearly unchanged. The nuclei are similarly variable, with slight changes in the relative volume. In the stunted rats refed one or two weeks, the cells and nuclei in general approach, but have not yet reached, their normal size.

8. In adult acute inanition there is but little change in the size of the cells and nuclei in the outer and inner cortical zones. In the middle zone and medulla, however, there is a marked loss in size of the cells, though but slight loss in the nuclei (with corresponding increase in relative nuclear volume). In chronic adult inanition there is a greater decrease in the size of the outer- and middle-zone cells, but about the same as during acute inanition in the inner zone and medulla. The nuclear loss is somewhat greater than during acute inanition, with relative nuclear volume not very different from normal, except in the middle zone (where it is high).

9. Amitosis in the suprarenal of the rat is infrequent and of doubtful significance. Mitoses are frequent in the new-born (average about 20 per section), but fewer at the end of the first week (10 per section). They increase to a maximum rate during the second and third weeks, slowly decreasing in number thereafter, although occasionally found even in the adult. Mitoses are most frequent in the outer zone and outermost part of the middle zone (these forming the 'germinative zone,) less frequent in the medulla, and rare in the inner cortical zone.

10. In young rats stunted by underfeeding, mitosis is more or less completely suppressed in the suprarenal. On refeeding one week, mitosis begins again, and in two weeks the normal rate is reestablished. Cell division in the suprarenal is therefore controlled by the amount of nutrition.

11. The three cortical zones of the suprarenal are distinguishable from birth and well differentiated at three weeks. Liposomes (lipoidal granules or droplets) are present in considerable amount at birth, being rather uniformly distributed through the cortex and the cortical strands through the medulla. They increase slowly rather and the distribution changes. They become more abundant in the outer zone and outer half of the middle zone, decreasing in amount toward the inner zone, with none in the medulla. The inner cortical zone is atrophic in character, with cells in various stages of degeneration and absorption. Pigment (lipochrome) appears in these cells after eight weeks of age. The degeneration and absorption of this zone is associated with the expansion of the medulla in the morphogenesis of the suprarenal gland. The absorption of the inner cortical zone during the growth of the suprarenal is characteristic for mammals, though greatly exaggerated in the human infant. The chromaffin reaction of the medulla is weak at birth, becoming well marked in the rat after the age of three weeks.

12. In the young rats stunted by underfeeding certain cell changes occur in the suprarenal. The cortex tends to undergo the normal differentiation of liposomes, but their amount is variable. In some cases many of the cells appear atrophic and degenerative, especially toward the inner cortical zone, where the process is extreme. Pigment appears in unusual abundance. The medulla is usually less affected, although pycnotic nuclei frequently occur. Only in extreme cases is there any appreciable decrease in the chromaffin reaction. In stunted rats refed two weeks the structure is already gaining a normal appearance, though some areas of degeneration persist.

13. In adult rats subjected to acute or chronic inanition the suprarenal cells present a simple atrophy, together with a variable amount of degeneration. The nuclei may be either hypochromatic or hyperchromatic, with frequent pycnosis or karyolysis. The liopsomes are retained tenaciously in the outer zone, though usually decreased to a considerable extent in the rest of the cortex, especially in extreme inanition. The pigment remains unchanged. The medulla cells show degenerative changes,

though usually less marked than those of the cortex. The chromaffin reaction is apparently retained undiminished up to the point of death, though occurring as a postmortem change.

LITERATURE CITED

Babes, V., et Jonesco, V. 1908 Distribution de la graisse dans les capsules surrénales. C. R. Soc. Biol. Paris, T. 65, Ann. (1908, T. 2)

Bager, Bertel 1917 Bidrag till binjurarnas aldersanatomi hos kaninen. (Zur Altersanatomie der Nebennieren des Kaninchens.) Upsala Läkareförenings Förhandlingar. Ny. följd., Bd. 23, H. 1–2, S. 48-116.

Barbèra, A. G., e Bicci, D. 1900 Contributo istologico alla conoscenza delle modificazioni che il digiuno apporta negli elementi anatomici dei vari organi e tessuti del l'economia animal. Prima nota: Capsule soprarenali. Bollettino delle scienze mediche. SocietaMedico-Chirurgica e della Scuola Medica di Bologna. Anno 71, Serie 7, vol. 11, pp. 679–682.

Baroncini, L., e Beretta, A. 1901 Ricerche istologiche sulle modificazioni degli organi mammiferi ibernanti. IV. Capsule surrenali (Nota preventiva). Lo Riforma Medica, Anno 16, pp. 162–163; Anno 17, pp. 76–78.

Bell, E. T. 1910 The staining of fats in epithelium and muscle fibers. Anat. Rec., vol. 4, no. 5, pp. 199–212.

Beneke —— Pathologische Anatomie der Nebennieren, in Zülzer's Handbuch der Harn- und Geschlechtsorgane. (Cited by Ewald, '02.)

Biedl, A. 1913 Innere Sekretion. 2 Aufl., 2 Abth. (S. 16).

Bonnamour, S. 1905 a Étude histologique des phénomènes de secrétion de la capsule surrénale chez les mammifères. Thèse, Lyon.

1905 b Modifications histologiques de la capsule surrénale dans certains états physiologiques (hibernation, inanition) et pathologiques expérimentaux (diphthérie, rage). C. R. de l'Assoc. des Anatomistes (Int. Congr. Anat. Geneva, 1905), vol. 7, pp. 87–93.

Borberg 1912 Das chromaffine Gewebe. Nebennierenuntersuchungen. II. Skand. Archiv f. Physiol., Bd. 28, S. 91. (Cited by Biedl, '13.)

Canalis, P. 1887 Contribution à l'étude du développement et de la pathologie des capsules surrénales. Jour. internat. mens. d'anatomie et de physiol. (Int. Monatschr.), T. 4, pp. 312–333.

Ciaccio, C. 1903 Ricerche sui processi di secrezione cellulare nelle capsule surrenali dei vertebrati. Anat. Anz., Bd. 23, S. 401–424.

1905 Sur la fine structure et sur les fonctions des capsules surrénales des vertébrés. Arch. ital. de biol. T., 43.

1910 Contributo alla distribuzione ed alla fisio-patologia cellulare dei lipoidi. Arch. f. Zellforschung, Bd. 5, S. 235–363.

Cottentot, Mulon, et Zimmern 1912 Action des rayons X sur la corticale surrénale. C. R. Soc. Biol. Paris, T. 73, pp. 717-720.

Cristiani, M. et Mme. 1902 Histologie pathologique des greffes de capsules surrénales. C. R. Soc. Biol. Paris, T. 54, pp. 811–814.

Da Costa, A. C. 1913 Recherches sur l'histo-physiologie des glandes surrénales. Archives de Biol., T. 28, p. 111–196.

Delamare, G. 1903 Recherches sur la sénescence de la glande surrénale. C. R. Soc. Biol. Paris, T. 55, pp. 1152–1154.

Dewitzky, W. 1912 Beiträge zur Histologie der Nebennieren. Ziegler's Beiträge, Bd. 52, S. 431–443.

Donaldson, H. H. 1915 The rat. Reference tables and data. Memoirs of The Wistar Institute, no. 6, Philadelphia.

Dostoiewsky, A. 1886 Ein Beitrag zur mikroskopischen Anatomie der Nebennieren bei Säugethieren. Archiv f. mikr. Anat., Bd. 27, S. 272–296.

Elliott, T. R., and Armour, R. G. 1911 The development of the cortex in the human suprarenal gland and its condition in hemicephaly. Jour. of Path. and Bact., vol. 15, pp. 481–488.

Elliott, T. R., and Tuckett, I. 1906 Cortex and medulla in the suprarenal glands. Jour. Physiol., vol. 34, no. 4–5.

Ewald, Paul 1902 Über Fettgehalt und multiple Adenombildung in der Nebenniere. Inaug.-Dissert. München.

Flint, J. M. 1900 The blood-vessels, angiogenesis, organgogenesis, reticulum and histology of the adrenal. Johns Hopkins Hosp. Rep., vol. 9, pp. 153–230.

Frederici 1903 Lo sperimentale (cited, without title, by Ciaccio, '10).

Gottschau, M. 1883 Structur und embryonale Entwickelung der Nebennieren bei Säugethieren. Archiv f. Anat. u. Physiol., Anat. Abth., S. 412–458.

Graham, G. S. 1916 Toxic lesions of the adrenal gland and their repair. Jour. Med. Research, vol. 34, pp. 241–261.

Hatai, S. 1913 On the weights of the abdominal and the thoracic viscera, the sex glands, ductless glands and the eyeballs of the albino rat (Mus norvegicus albinus) according to body weight. Am. Jour. Anat., vol. 15, no. 1.

Herman 1905 Über Vorkommen und Veränderungen von Myelinsubstanz in der Nebenniere. Inaug.-Dissert. Tübingen. (Cited by Kawamura, '11.)

Hornowski, J. 1909 Veränderungen im Chromaffinsystem bei unaufgeklärten postoperativen Todesfällen. Virchow's Archiv, Bd. 198.

Hoskins, E. R. 1916 The growth of the body and organs of the albino rat as affected by feeding various ductless glands (thyroid, thymus, hypophysis and pineal). Jour. Exper. Zool., vol. 21, no. 3.

Hultgren und Anderson 1899 Studien über die Physiologie und Anatomie der Nebennieren. Skand. Arch. f. Physiol., Bd. 9, S. 73–311.

Inaba, M. 1891 Notes on the development of the suprarenal bodies in the mouse. Jour. College of Science, Imp. Univ. Japan, vol. 4, part 1, pp. 215–235.

JACKSON, C. M. 1913 Postnatal growth and variability of the body and of the various organs in the albino rat. Am. Jour. Anat., vol. 15, pp. 1-68.

1915 a Effects of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. Am. Jour. Anat., vol. 18, pp. 75–116.

1915 b Changes in the relative weights of the various parts, systems and organs of young albino rats held at constant body weight by underfeeding for various periods. Jour. Exper. Zool., vol. 19, pp. 99–156.

1917 Effects of inanition and refeeding upon the growth and structure of the hypophysis in the albino rat. Am. Jour. Anat., vol. 21, pp. 321–358.

KAWAMURA, R. 1911 Die Cholesterinesterverfettung (Cholesterinsteatose). Ein differentialdiagnostische morphologische Studie über die in den menschlichen und tierischen Geweben vorkommenden Lipoide. Verlag G. Fischer, Jena.

KEIBEL, F. 1904 Zoologische Forschungsreisen in Australien und dem Malayischen Archipel von Richard Semon, Bd. 3. Monotremen und Marsupialer, II. 2. Teil, S. 151–206. (Cited by Poll, '05.)

KERN, H. 1911 Über den Umbau der Nebenniere im extrauterinen Leben. Deutsche med. Wochenschr., Bd. 37, S. 971–974; 1180; 1318–1319.

KOLMER, W. 1912 a Über gewisse physiologisch-histologische Vorgänge in der Nebenniere und deren Beziehung zum Genitalapparat. Zentralbl. f. Physiol., Bd. 25, S. 1009.

1912 b Beziehung von Nebenniere und Geschlechtsfunktion. Pflüger's Archiv, Bd. 144, S. 361–395.

KURIYAMA, S. 1918 The adrenals in relation to carbohydrate metabolism. III. The epinephrin content of the adrenals in various experimental conditions. Jour. Biol. Chem., vol. 34, pp. 299–319.

LANDAU, M. 1913 a Zur Entwicklung der Nebennierenrinde. Deutsche med. Wochenschr., Bd. 39, no. 7, Feb. 13.

1913 b Nebenniere und Fettstoffwechsel. Deutsche med. Wochenschr., Bd. 39, no. 12, May 20.

LEWIS, R. W., AND PAPPENHEIMER, A. M. 1916 A study of the involutional changes which occur in the adrenal cortex during infancy. Jour. Med. Research, vol. 34, pp. 81–93.

LUKSCH, F. 1911 Über das histologische und funktionelle Verhalten der Nebennieren beim hungernden Kaninchen. Arch. f. exper. Path. u. Pharm., Bd. 65, S. 161–163.

MANN, F. C. 1916 The ductless glands and hibernation. Amer. Jour. Physiol. vol. 41, no. 2.

MARTINOTTI, C. 1892 Contributo allo studio delle capsule surrenale. Giornale della R. Accad. di Med. di Torino. Serie III., vol. 40, pp. 299–301.

MAYER, MULON, ET SCHAEFFER 1912 Contribution à la microchemie des surrénales. I. Recherches sur les surrénales du cheval. C.. R. Soc. Biol. Paris, T. 73, pp. 313–315. II. Recherches sur les surrénales de mouton. Ibid., pp. 315–318.

Meyer, A. W. 1917 Some morphological effects of prolonged inanition. Jour. Med. Research, vol. 36, pp. 51–78.

Minot, C. S. 1897 Human embryology. New York.

Mulon, P. 1902 Excretion des capsules surrénales du cobaye dans les vaisseaux sanguins. C. R. Soc. Biol. Paris, T. 54, pp. 1540–1542.

1903 a Sur le pigment des capsules surrénales chez le cobaye. C. R. de l'Assoc. des Anat., T. 5, pp. 143–151.

1903 b Divisions nucléaires et rôle germinatif de la couche glomérulaire des capsules surrénales du cobaye. C. R. Soc. Biol., Paris, T. 55, pp. 592–595.

1905 a Évolution de la corticale surrénale du cobaye aves l'age de l'animal. C. R. Soc. Biol. Paris, T. 59 (1905, T. 2), pp. 337–339. (Cf. also pp. 592–593.)

1905 b Sur le pigment des capsules surrénales (Cobaye). Bibl. Anat. T. 14, pp. 177–182.

1912 La corticale surrénale du chien. C. R. Soc. Biol. Paris, T. 73, pp. 714–716.

Napp, O. 1905 Über den Fettgehalt der Nebenniere. Virchow's Archiv, Bd. 182, S. 314–326.

Orth, J. 1893 Lehrbuch der path. Anat., Bd. 2.

Pellegrini, R. 1916 (Effect of fasting on the suprarenals.) Atti r. Inst. Veneto, vol. 72, p. 781. (Cited from 'Chemical Abstracts' in Endocrinology, vol. 1, no. 3, 1917, p. 354.)

Pellegrino, M. 1904 Sopra una particulare disposizione della sostanze midollare nelle capsule surrenali (mammiferi). Bollet. d. Soc. di Nat. in Napoli, Anno 18, Série I, pp. 139–142. (Cited by Poll, '05, and Da Costa, '13.)

Pfaundler, M. 1892 Zur Anatomie der Nebenniere. Sitzungsber. d. k. Akad. d. Wiss. in Wien. Math.-Naturw. Classe, Bd. 101, Abth. 3, S. 515–533.

Poll, H. 1899 Veränderungen der Nebenniere bei Transplantation. Archiv f. mikr. Anat., Bd. 54, p. 440–481.

1905 Die Entwickelung der Nebennierensysteme, in Hertwig's Handbuch der Entwickelungslehre der Wirbeltiere. Bd. 3, Teil 1.

Ponomarew, A. 1914 Über den Ursprung der Fettsubstanzen in der Nebennierenrinde. Ziegler's Beiträge, Bd. 59, H. 2. (Reviewed in Zentralblatt f. norm. Anat. u. Mikrotechnik, Bd. 11, H. 12, 1914.)

Rondoni, P. e Montagnani, M. 1915 Lesioni istologiche nel maidismo, nel digiuno e nello scorbuto sperimentale. Lo sperimentale, Anno 69, pp. 659–696.

Roud, Aug. 1902 Contribution à l'étude du développement de la capsule surrénale de la souris. Bull. de la soc. vaudoise des sc. nat., T. 38, pp. 187–258. (Cited from Jahresbericht f. Anat., volume for 1903, and Poll, '05.)

Scheel, O. 1908 Über Nebennieren. Sekretkörnchen-Oedem-Gewicht. Virchow's Archiv, Bd. 192, S. 494–513.

Schur, H., und Wiesel, J. 1908 Über das Verhalten des chromaffinen Gewebes bei der Narcose. Wiener klin. Wochenschr., Jahrg. 21, No. 8.

SOULIÉ, A. H. 1903 Recherches sur le développement des capsules surrénales chez les vertébrés supérieurs. Jour. de l'anat. et de la physiol., T. 39, pp. 197–293; 492–533; 634–662.

STARKEL, S., UND WEGRZYNOWSKI, L. 1910 Beitrag zur Histologie der Nebenniere bei Feten und Kindern. Arch. f. Anat. u. Physiol., Anat. Abth., S. 214–326. (Also (Polish) Medycyna Warschau, reviewed by Hoyer in Jahresbericht f. Anat. for 1910, III, p. 461–462.)

STEWART, C. A. 1916 Growth of the body and of the various organs of young albino rats after inanition for various periods. Biol. Bull., vol. 31, pp. 16–51.

THOMAS, E. 1911 Über die Nebenniere des Kindes und ihre Veränderungen bei Infektionskrankheiten. Ziegler's Beiträge, Bd. 50, S. 283–316.

TRAINA, R. 1904 Über das Verhalten des Fettes und der Zellgranula bei chronischem Marasmus und akuten Hungerszuständen. Ziegler's Beiträge, Bd. 35, S. 1–92.

VENULET, F., UND DMITROWSKY, G. 1910 Über das Verhalten der chromaffinen Substanz der Nebennieren beim Hungern und unter dem Einfluss von Iodkali. Archiv. f. exper. Path. u. Pharm., Bd. 63, S. 460–464.

WIESEL, J. 1901 Über die Entwicklung der Nebenniere des Schweines, besonders der Marksubstanz. Anat. Hefte, Bd. 16, S. 117–150.
1902 Zur Entwickelung der menschlichen Nebenniere. Centralbl. f. Phys., Bd. 15, Verh. d. Morphol.-Physiol. Gesellsch. zu Wien (1901), S. 614–615.

ZUCKERKANDL, E. 1912 The development of the chromaffin organs and of the suprarenal glands. In Kiebel and Mall's Human Embryology, vol. 2.

TABLE 1

Individual number, age and condition, sex, body length, body weight and weight of the suprarenal glands in the albino rats used for histological study

RAT NO.	AGE, ETC.	SEX	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (OR NET)	WEIGHT OF SUPRARENALS	SEE NOTE
A. Normal rats						
			mm.	*grams*	*grams*	
F 10.1	New-born	m.	46	4.2	0.0014	*
F 10.2	1 day	m.	50	4.8	0.0014	*
J 1.7a	New-born	m.		4.9		
J 1.7b	New-born	m.		4.9		
F 8.1	New-born	m.	50	5.1	0.0018	*
F 11.1	3 days	m.	57	6.5	0.0020	*
F 11.2	5 days	f.	60	8.3	0.0020	*
F 11.3	8 days	m.	64	9.6	0.0018	*
St 72.5	7 days	f.	66	10.8 (10.1)	0.0024	
St 72.2	7 days	m.	66	10.8 (10.0)	0.0024	
V 13.1	10 days	m.		12.0	0.0030	
St 80.5	12 days	f.	75	15.5	0.0040	
V 1.3	14 days	m.	75	15.4		
F 7.1	17 days	f.	86	26.3	0.0054	*
V 1.4	21 days	m.	87	20.4		
S 5.2	21 days	f.	95	22.9 (21.1)	0.0100	
J 1.2	21 days	f.	100	28.2	0.0078	
J 1.1	21 days	m.	100	29.0	0.0078	
St 47.2	21 days	m.	102	34.3	0.0134	
St 5.1	56 days	f.		63.0	0.0116	
St 5.2	56 days	m.		77.0	0.0182	
St 228.2	58 days	m.	156	96.0	0.0183	*
St 47.6	67 days	f.	169	124.0	0.0364	
St 47.5	67 days	m.	191	196.0	0.0326	
H 70.3	70 days	m.	194	208.0	0.0357	
H 68.11	72 days	m.	192	171.0 (164)	0.0228	
H 68.8	74 days	f.	184	142.0 (137)	0.0339	
S 5.4	74 days	f.	168	126.0 (118)	0.0319	
S 5.3	74 days	m.	180	172.0 (167)	0.0289	
M 1.2	74 days	m.	190	181.0 (173)	0.0264	
J 1.3	94 days	m.	183	177.0	0.0249	
H 64.3	101 days	m.	191	185.0 (179)	0.0264	
H 60.7	103 days	f.	203	(182)	0.0302	
F 5.1	105 days	m.	188	192.0	0.0234	*
H 58.3	106 days	m.	225	(258)	0.0404	
J 1.7	112 days	f.	185	161.0	0.0365	(1)
F 2.1	116 days	m.	176	149.0	0.0268	*
Mo. 9	120 days	f.	180	157.0		
H 36.3	138 days	m.	202	(202)	0.0406	
H 50.3	141 days	m.	211	(222)	0.0406	

TABLE 1—*Continued*

RAT NO.	AGE, ETC.	SEX	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (OR NET)	WEIGHT OF SUPRA-RENALS	SEE NOTE
			mm.	*grams*	*grams*	
F 1.1	141 days	m.	195	200.0	0.0234	*
F 9.1	170 days	m.	185	173.0	0.0246	*
F 6.1	150 days	m.	188	178.0	0.0290	*
S 14	(adult)	m.	205	252.0 (247)	0.0414	
H 21.3	202 days	m.	216	(232)	0.0304	
H 34.3	224 days	f.	194	(173)	0.0453	
H 34.6	225 days	f.	205	(188)	0.0604	
L 3.7	234 days	m.	190	241.0 (238)	0.0310	
H 27.3	253 days	f.	195	(166)	0.0475	
H 27.6	254 days	m.	214	(222)	0.0298	
S 33.116	340 days	f.	195	194.0 (188)	0.0414	
S 33.117	346 days	m.	228	302.0 (294)	0.0330	
B. Stunted young rats						
St 247.5	Maint. 1–7 days	f.	53	4.5	0.0016	*(2)
St 80.9	Underfed 1–12 days	f.	64	8.9	0.0026	(2)
St 228.4	Underfed 1–58 days	m.	74	10.9	0.0054	*(2)
St 9.36	Maint. 21–51 days	m.	113	30.5	0.0124	(2)
S 12.69	Maint. 21–66 days	f.	100	24.5 (22.7)	0.0090	(2)
St 47.4	Maint. 21–66 days	m.	113	32.3	0.0140	(2)
St 47.3	Maint. 21–66 days	m.	120	34.0	0.0136	(2)
S 12.71	Maint. 21–67 days	m.	95	23.3 (21.2)	0.0086	(2)
S 7.34	Maint. 21–70 days	m.	115	30.5 (29.0)	0.0116	(2)
S 7.31	Maint. 21–70 days	m.	120	34.8 (31.4)	0.0126	(2)
S 7.32	Maint. 21–71 days	m.	120	30.0 (28.8)	0.0100	(2)
S 7.35	Maint. 21–71 days	f.	117	35.3 (31.0)	0.0120	(2)
S 11.65	Maint. 21–73 days	m.	100	23.8 (22.5)	0.0118	(2)
St 12.50	Maint. 21–82 days	m.	123	45.0 (41.2)		(2)
St 33.1	Maint. 22–104 days	f.	89	19.1 (18.2)	0.0086	(2)
St 38.8	Maint. 21–139 days	m.	118	30.0	0.0161	(2)
C. Refed after inanition						
St 12.48	88 days Refed 7 days	f.	125	50.2 (46.5)	0.0105	(3)
St 11.42	88 days Refed 7 days	f.	125	55.0 (50.3)	0.0160	(3)
St 11.45	88 days Refed 7 days	f.	130	67.6 (57.7)	0.0156	(3)
St 10.27	89 days Refed 7 days	f.	127	55.0 (51.4)	0.0115	(3)
St 12.51	95 days Refed 14 days	f.	137	77.2 (70.7)	0.0150	(3)
St 11.43	95 days Refed 14 days	f.	143	79.0 (74.7)	0.0196	(3)
St 11.40	95 days Refed 14 days	f.	142	84.5 (76.5)	0.0158	(3)
St 10.26	96 days Refed 14 days	m.	150	91.0 (86.3)	0.0180	(3)
F 2.3	122 days Refed 6 days	m.	176	144.0	0.0350	*(3)
S 33.120	339 days Refed 189 days	f.	181	162.0 (156)	0.0396	(3)
S 33.118	346 days Refed 206 days	m.	204	229.0 (219)	0.0348	(3)

TABLE 1—*Concluded*

RAT NO.	AGE, ETC.	SEX	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (OR NET)	WEIGHT OF SUPRA-RENALS	SEE NOTE
			D. Adult acute inanition			
			mm.	*grams*	*grams*	
F 2.2	116 days (loss 29%)	m.	173	104.0	0.0316	*(4)
F 1.2	147 days (loss 30%)	m.	188	162.0	0.0374	*(4)
S 27	(loss 30%)	m.	215	223.0 (219)	0.0424	(4)
M 2	(loss 33%)	m.	185 (?)	170.0 (167)	0.0338	(4)
F 9.3	170 days (loss 33%)	m.	179	129.0	0.0334	*(4)
F 6.2	151 days (loss 34%)	m.	183	114.0	0.0422	*(4)
F 3.2	130 days (loss 34%)	m.	171	115.0	0.0280	*(4)
J 1.4	94 days (loss 35%)	m.	180	107.0	0.0302	(4)
S 25	(loss 35%)	m.	205	202.0 (198)	0.0458	(4)
F 9.2	170 days (loss 36%)	m.	191	127.0	0.0374	*(4)
S 26	(loss 39%)	m.	205	174.0 (171)	0.0233	(4)
F 4.2	153 days (loss 43%)	m.	172	106.0	0.0332	*(4)
F 5.2	105 days (loss 45%)	m.	185	110.0	0.0318	*(4)
			E. Adult chronic inanition			
J 1.5	117 days (loss 34%)	m.	175	97.0	0.0240	(5)
M 3	(loss 34%)	m.	175	125.0 (122)	0.0252	(5)
M 12	(loss 36%)	m.	173	128.0 (125)	0.0306	(5)
M 5	(loss 37%)	m.	190	129.0 (127)	0.0320	(5)
M 6	(loss 37%)	m.	175	138.0 (134)	0.0270	(5)
M 11	(loss 38%)	m.	190	163.0 (159)	0.0322	(5)

* The 'F' series, and a few others as indicated, were cut by frozen sections for study in the fresh condition, or for lipoids, chromaffin reaction, etc. All of the remaining glands were embedded and cut in paraffin sections.

[1] Rat J 1.7 had just given birth to a (first) litter. The remaining females were virgins.

[2] Of the stunted rats (table 1 B), St 80.9 and St 228.4 had practically doubled their initial weight during the period of underfeeding. The others were held nearly at maintenance (constant body weight) during the period indicated.

[3] Of the rats refed after inanition (table 1 C), all excepting the last three were refed after having been held nearly at maintenance from three to about twelve weeks of age. S 33.118 and S 33.120 had been held with but slight increase in body weight from 21 to 140 and 150 days of age, respectively, and were apparently permanently stunted. Rat F 2.3 decreased from 151 grams to 104 grams (loss of 31 per cent) during seven days of acute inanition, and was then refed as indicated.

[4] The rats subjected to acute inanition (table 1 D) had been given water only for periods varying from seven to twelve days, with loss in body weight as indicated. In four cases the age was unknown. The final body weights are given in the table.

[5] The rats with chronic inanition (table 1 E) had been fed gradually decreasing amounts of food during a period of about five weeks, with losses in body weight as indicated. The final body weights are given. The age was unknown in all but the first rat.

TABLE 2

Postnatal growth of volume in suprarenal cortex and medulla in the albino rat

AGE AND CONDITION	NUMBER[1] AND SEX	GROSS BODY WEIGHT AVERAGE	SUPRARENALS WEIGHT AVERAGE	SUPRARENALS PER CENT OF BODY WEIGHT AVERAGE	PERCENTAGE FORMED BY THE	
					Cortex average	Medulla, average (and range)
A. Normal						
		grams	*grams*	*per cent*	*per cent*	*per cent*
7 days	1 m	10.8	0.0024	0.025	74.5	25.5
7 days	2 f	10.8	0.0024	0.023	79.8	20.4 (18.6–21.7)
10–14 days	3 m	13.7	(0.0031)	(0.019)	80.9	19.1 (16.8–22.3)
10–14 days	2 f	15.5	0.0040	0.026	84.3	15.7 (15.3–16.4)
21 days	5 m	27.9	0.0106	0.033	90.6	9.4 (8.5–10.8)
21 days	2 f	28.2	0.0078	0.028	89.5	10.5 (10.0–11.0)
56 days	2 m	77.0	0.0182	0.024	92.3	7.7 (7.4–8.1)
56 days	1 f	63.0	0.0116	0.018	82.7	17.3
67–74 days	3 m	180.3	0.0218	0.012	92.8	7.2 (5.8–8.0)
67–74 days	3 f	132.6	0.0414	0.032	93.5	6.5 (5.3–7.2)
94–346 days	8 m	220.1	0.0322	0.015	89.9	10.1 (6–16)
112–253 days	4 f	161.1	0.0426	0.027	90.9	9.1 (7.0–11.9)
B. Effect of experiments						
Underfed 1–12 days	2 f	8.9	0.0026	0.029	79.3	20.6 (18.6–22.7)
Maintenance 21–51 days	1 m	30.5	0.0124	0.040	90.2	9.8
Maintenance 3–10 weeks	7 m 1 f	34.1	0.0131	0.041	89.8	10.2 (8.7–12.5)
Maintenance 21–82 days	2 m	45.0			88.2	11.8 (11.4–12.2)
Maintenance 21–139 days	1 m	30.0	0.0161	0.054	89.3	10.7
Refed 1 week	6 f	57.6	0.0125	0.024	89.4	10.6 (9.6–11.6)
Refed 2 weeks	1 m 5 f	82.9	0.0171	0.022	90.4	9.6 (8.8–11.1)
Refed 189 days	2 f	162.0	0.0396	0.024	85.7	14.3 (13.4–15.3)
Refed 206 days	2 m	229.0	0.0348	0.016	90.8	9.2 (9.0–9.4)
Adult acute inanition	6 m	177.3	0.0393	0.024	90.1	9.9 (5.8–12.8)
Adult chronic inanition	9 m	123.4	0.0278	0.023	88.7	11.3 (8.1–13.1)

[1] The 'No.' in the second column refers to number of glands, not animals. The suprarenal weights were missing in one of the three normal males at ten to fourteen days.

TABLE 3

Average relative volumes of the stroma and vessels in the various zones of the cortex and medulla in the suprarenal gland of the albino rat

AGE AND CONDITION	NUMBER OF GLANDS	AVERAGE PERCENTAGE OF VASCULAR STROMA IN ZONES OF SUPRARENAL CORTEX				
		Outer zone	Middle (outer half)	Middle (inner half)	Inner zone	Medulla
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Normal 1–14 days......	3	14.4 (12.1–18.5)	8.4 (6–9.4)	8.4 (6–9.4)[1]	18.1 (13.4–18.7)	32.8 (27.–37.6)
Normal 3 weeks to adult................	5	13.2 (11.1–15.0)	8.6 (5.8–13.5)	12.5 (10–16.7)	19.1 (10.4–23.3)	27.7 (19.8–33.5)
Maintenance from 3 weeks................	5	10.6 (8.9–12.8)	6.4 (4.4–8.6)	9.0 (7.5–10.4)	28.0 (16.7–39.9)	29.7 (16.2–44.1)
Refed 1 week after maintenance.........	2	6.2 (5.1–7.4)	9.2 (8.6–9.8)	10.4 (7.2–13.6)	15.6 (12.5–18.6)	19.9 (12.4–27.4)
Refed two weeks after maintenance.........	2	9.3 (7.8–10.8)	14.3 (8.4–20.3)	9.6 (8.4–10.8)	21.1 (18–24.3)	23.4 (22.5–24.2)
Adult acute inanition..	4	12.4 (6.4–15.2)	12.6 (8.4–18.1)	16.9 (13.1–26.4)	22.2 (14.0–30.8)	23.5 (16.5–36)
Adult chronic inanition	2	12.4 (12.3–12.6)	13.5 (10–17.1)	24.6 (23–26.2)	23.7 (21.8–25.6)	16.2 (12.4–20)

---

[1] In normal group of one to fourteen days the outer and inner portions of the middle zone were not measured separately.

TABLE 5

Mitoses in the suprarenal gland of the albino rat

AGE AND CONDITION	NUMBER OF GLANDS	AVERAGE NUMBER OF MITOSES PER SECTION IN				
		Outer zone	Middle zone	Inner zone	Medulla	Total average (and range)
A. In normal rats						
New-born	3	11	2	1	6	20 (12–29)
7 days	3	5	2	$\frac{1}{3}$	3	10 (7–13)
10 days	2	6	5	$\frac{1}{2}$	4	16 (14–18)
12 days	1	18	17	0	5	40
14 days	2	10	10	$\frac{1}{2}$	6	27 (22–31)
21 days	9	9	12	$\frac{4}{9}$	2	24 (10–41)
56 days	1	3	5	0	3	11
67–94 days	4	2	2	$\frac{1}{4}$	0	4 (2–5)
112–138 days	2	1	1	0	0	2 (1–3)
340 days	1	1	0	0	0	1
B. In test rats						
Maintenance birth to 12 days	1	1	1	0	2	4
Maintenance 3–10 weeks	10[1]	$\frac{3}{5}$	$\frac{1}{2}$	0	0	1 (0–3)
Maintenance 3–12 to 20 weeks	3	0	0	0	0	0
Refed 1 week	3	$\frac{1}{3}$	1	0	$\frac{1}{3}$	2 (1–3)
Refed 2 weeks	2	3	5	0	2	10 (7–12)
Refed to 1 year	1	0	0	0	0	0

[1] Six of the ten held at maintenance from three to ten weeks of age showed no mitoses in the sections examined.

TABLE 4

Absolute and relative size of cells and nuclei in the suprarenal parenchyma of the albino rat

RAT NO	CONDITION AND AGE	AVERAGE CELL DIAMETER					AVERAGE NUCLEAR DIAMETER					RELATIVE VOLUME OF NUCLEUS				
		Outer zone	Middle zone		Inner zone	Medulla	Outer zone	Middle zone		Inner zone	Medulla	Outer zone	Middle zone		Inner zone	Medulla
			Outer	Inner				Outer	Inner				Outer	Inner		
		μ	μ	μ	μ	μ	μ	μ	μ	μ	μ	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
J 1.7a	Normal newborn	7.1	8.9	9.3	9.4	7.9	5.4	6.2	6.1	6.1	6.1	44.0	33.1	28.2	27.8	46.0
St 72.2	Normal 1 week	6.7	9.4	9.0	8.5	10.7	4.6	6.0	5.6	5.3	6.0	32.2	25.7	24.0	23.5	17.8
V 1.3	Normal 2 weeks	7.7	9.7	10.3	9.2	10.2	4.9	5.6	5.8	5.4	6.5	25.8	19.0	17.5	20.0	25.6
J 1.1	Normal 3 weeks	7.7	9.7	10.3	9.8	10.4	5.5	6.2	6.2	5.8	6.6	35.6	26.3	21.3	21.0	25.5
J 1.2	Normal 3 weeks	8.3	9.6	10.7	9.2	11.3	5.7	6.2	6.3	5.6	6.8	32.5	27.0	20.6	22.9	21.6
St 47.5	Normal 10 weeks	9.0	14.1	11.5	8.2	12.8	5.8	7.3	6.4	5.0	7.4	25.8	13.8	17.5	21.9	19.3
St 47.6	Normal 10 weeks	8.3	14.6	12.2	10.2	13.6	5.4	6.9	6.4	5.6	7.0	26.8	10.7	14.5	16.5	13.3
S 33.117	Normal 1 year	9.0	14.8	11.5	8.7	15.9	5.5	6.9	6.2	5.2	7.3	23.2	10.1	15.5	21.4	9.6
St 47.3	Maintenance 21–66 days	8.6	10.9	9.4	8.8	11.5	5.5	5.8	5.7	5.2	6.4	25.8	14.9	23.1	20.6	17.0
S 7.35	Maintenance 21–70 days	8.5	10.9	8.9	7.1	12.2	5.4	5.9	5.0	4.2	6.7	25.6	15.4	18.3	21.1	16.1
S 7.31	Maintenance 21–70 days	7.8	10.3	8.6	8.9	11.6	5.3	5.8	5.6	5.4	6.3	30.6	17.6	27.0	22.0	15.8
St 12.50	Maintenance 21–82 days	7.8	13.0	8.9	7.7	11.4	4.8	7.3	5.7	4.9	6.6	24.0	17.8	26.3	25.3	19.2
St 38.8	Maintenance 21–139 days	8.8	10.2	8.9	7.9	10.4	5.4	5.8	5.3	4.7	5.9	22.6	18.7	20.6	21.3	17.8
Average for group		8.1	11.1	8.9	8.1	11.4	5.3	6.1	5.5	4.9	6.4	25.7	16.9	23.1	22.1	17.2
St 11.45	Refed 1 week	8.3	11.3	8.5	8.9	12.5	5.1	5.8	5.1	5.1	6.4	22.9	13.3	21.8	18.6	13.5
St 10.27	Refed 1 week	8.9	12.3	9.7	9.6	12.0	5.4	6.0	5.5	5.6	5.7	21.8	11.8	18.0	19.8	10.7
St 10.26	Refed 2 weeks	8.8	13.4	9.7	9.3	11.6	5.3	6.0	5.4	5.3	6.2	22.0	8.9	17.4	18.5	15.2
St 11.43	Refed 2 weeks	8.6	12.8	9.8	8.9	12.5	5.3	6.6	5.7	5.4	6.2	23.1	13.5	19.7	22.7	12.0
Average for group		8.7	12.5	9.4	9.2	12.2	5.3	6.1	5.4	5.4	6.1	22.5	11.9	19.2	19.9	12.9

S 27	Adult acute inanition	9.9	11.8	9.0	8.3	13.7	6.1	6.4	5.6	5.2	6.7	23.1	16.0	23.5	24.5	11.9
J 1.4	Adult acute inanition	8.7	11.3	8.7	8.0	11.7	6.2	6.7	5.3	5.0	7.2	35.6	20.9	23.1	24.4	22.8
M 2	Adult acute inanition	8.7	13.2	9.3	9.1	13.8	5.5	6.6	5.5	5.4	7.2	25.8	12.2	20.9	20.8	14.0
S 26	Adult acute inanition	8.4	11.7	9.9	8.7	11.7	5.2	6.3	5.7	5.1	6.7	23.9	15.7	19.3	20.2	18.3
Average for group		8.9	12.0	9.2	8.5	12.7	5.8	6.5	5.5	5.2	7.0	27.1	16.2	21.7	22.5	16.8
J 1.5	Adult chronic inanition	7.9	11.4	8.5	8.4	13.8	5.1	6.7	5.4	4.9	6.5	26.5	21.7	25.0	20.0	10.4
M 3	Adult chronic inanition	9.8	11.5	9.1	8.7	13.6	5.6	5.8	4.7	4.8	6.4	18.6	13.0	13.7	16.3	10.1
Average for group		8.6	11.5	8.8	8.6	13.7	5.4	6.3	5.1	4.9	6.5	22.6	17.4	19.4	18.2	10.3

No. 4

SKILL AND SCHOLARSHIP AS IDEALS IN MEDICAL GRADUATE EDUCATION *

C. M. JACKSON, M.S., M.D.
MINNEAPOLIS

Even in ancient times, physicians recognized the advantages of a division of labor, allowing concentration of effort in a relatively narrow field of medicine. The ancient Babylonians, according to Garrison, had a physician for every disease, and in the Code Hammurabi (2250 B. C.) medical fees were already regulated by law. Likewise among the Egyptians, as shown by the Ebers papyrus, medicine was highly specialized as early as 1550 B. C. Herodotus states that in the fifth century B. C. (in Egypt):

> Each physician applies himself to one disease only, and not more. All places abound in physicians; some physicians are for the eyes, others for the head, others for the teeth, others for the intestines, and others for internal disorders.

Although medical specialism was thus practiced by the ancients, who undoubtedly developed a high degree of skill in certain respects, for example, in operations for cataract by the Hindus, it soon reached its limits within comparatively narrow bounds. Throughout the ages, down to modern times, specialism, like medicine in general, made relatively slow progress, and for the same reason. The art of medicine necessarily remained empiric, awaiting the development of the fundamental sciences, physics, chemistry and biology. Before this, it was impossible for medicine to make real and rapid progress as an applied science.

With the rapid development of medicine during the past century, specialism has become increasingly nec-

---

* Read at a meeting of the staff and students of the Mayo Foundation for Medical Education and Research at Rochester, Minn., April 23, 1919.

essary, since no man could possibly cover thoroughly more than a small portion of this immense field. While contributing greatly to the advancement of medicine, however, the increasing tendency to specialism in clinical medicine carries with it certain obvious disadvantages and dangers. From the point of view of the individual specialist, the tendency to narrowness through isolation is evident. From the point of view of the general public, the cost of medical service by isolated specialists is becoming burdensome, if not prohibitive.

These difficulties, both from the professional and from the economic point of view, present a serious problem for consideration. The most promising solution appears to be the group system, whereby a staff of specialists may cooperate to practice medicine more efficiently. This means team-work associated with specialism, a principle which has been clearly realized and successfully carried out only in recent times. The Mayo Clinic is generally recognized as a pioneer in this work. The same principle has been followed with success in many other clinics, both public and private, and the movement is extending rapidly.

The tendency toward the group system in medical practice will doubtless receive an immense impetus from the experience during the war, as has been emphasized recently by Dodson. The excellent results obtained by this system in the numerous military and naval hospitals is an object lesson on a scale hitherto unknown. The thousands of medical practitioners who have thereby learned the increased efficiency of military medicine under this plan will doubtless desire to continue it on returning to civil practice. On the other hand, the advantages of the plan will not be lost on the millions of soldiers and sailors, who will naturally demand similar provision for medical service on their return to civil life. We may therefore expect in the near future to see medical groups organized for this purpose in every community able to support a hospital.

It is unquestionably true that preventive medicine will become increasingly prevalent, but this will by no means tend to minimize the importance of the group system. Public hygiene and sanitation likewise can be administered best by an organization of experts in various lines who must cooperate in the solution of the problems of preventive medicine. Moreover, by no means all forms of disease are preventable, even in theory. Curative medicine must therefore remain important, whether under private or state administration, and will certainly reach its greatest efficiency under the group system of organization.

Since well trained specialists are essential to the success of this plan, we may anticipate an increasing demand for them in the near future. Here also the war has revealed our present deficiencies. As Munson, Bispham and others have noted, a large proportion of our alleged specialists were found more or less incompetent in the military medical service.

Our present supply of real specialists thus is far below that indicated by the medical directory. In ophthalmology, for example, according to data cited by Reber, there are now in the United States about 5,000 listed ophthalmologists, of whom perhaps one fourth are really competent specialists. Edward Jackson estimates that about 10,000 trained ophthalmologists are needed in this country, or one to every 10,000 of population. Even if this number of ophthalmologists were now available, it is estimated that about 400 new men should be added each year in order to keep up the supply. The demand for well trained specialists in other lines is equally great.

To meet this demand, how are the specialists to be trained? In the first place, the plan of gradually narrowing from a general practice to a special field, a favorite method in the past, is now recognized as totally inadequate, unless supplemented by special training. For this special training, the opportunities offered by hospitals must be considered. Those hospitals with sufficient clinical facilities and with staffs efficiently organized are able to offer attractive educational opportunities to younger men who are willing to act as assistants during their period of apprenticeship. As has been pointed out by Welch, Barker and others, such positions as those of resident physician or surgeon in some of the larger hospitals represent, up to the present time, the best opportunities for capable young men who are ambitious to rise to eminence in the various phases of clinical medicine. Warner has recently emphasized the opportunities and responsibilities of the hospitals in the education of clinical specialists.

Unfortunately, such opportunities for hospital positions are now very limited in number, as well as defective in most cases from the educational point of view. This becomes apparent when one asks: "What training is needed for those who desire to attain leadership as clinical specialists in surgery, internal medicine, pediatrics, obstetrics, ophthalmology or other departments?" For the ideal education of specialists in medicine, the following conditions appear to me essential: (1) an adequate general medical education, including a hospital internship; (2) advanced work in the fundamental sciences; (3) advanced clinical instruction and training in the desired specialty; (4) training in research methods, and (5) adequate certification of proficiency. Let us consider these principles and the possibility of their realization under present conditions.

PRELIMINARY MEDICAL EDUCATION

An adequate general medical education is clearly an indispensable prerequisite for training in any branch of medical specialism. With a weak foundation, there can be no satisfactory superstructure. A graduate of a poor medical school cannot hope to become a competent specialist, unless he is willing first to go back and rebuild his primary medical education. Cases in which native genius is able to overleap the barrier of deficient preliminary education are rare.

Furthermore, a general hospital internship for at least a year, now recognized as an essential prerequisite for general practice, is even more important to give the broad, comprehensive clinical knowledge necessary for successful specialism. A narrow specialism will inevitably defeat its own ends. To avoid this obvious danger, the tradition still generally current in the medical profession requires several years of preliminary general practice. Recently, however, there has been marvelous improvement in clinical instruction in the better medical schools, making it possible (as Edward Jackson, Todd and others have pointed out) for the student to secure a better all-round clinical training by the end of his intern year than could formerly be obtained by several years of general practice.

There are certainly good educational and social reasons why the graduate medical training for specialism should begin as soon as possible. The graduate of five or ten years' standing has lost much of his mental elasticity, and usually finds it very difficult to resume serious and intensive study. A graduate of a first-class medical school, with a subsequent hospital internship, may therefore safely proceed at once to the desired line of specialization.

ADVANCED WORK IN THE FUNDAMENTAL SCIENCES

Following his hospital internship, the first step needed by the prospective specialist is to strengthen his knowledge of the fundamental sciences underlying the clinical application in his chosen field. This principle must be emphasized, as it is frequently overlooked or ignored.

As Bradford remarks in discussing the higher education in surgery:

> It has been said by those who undertake to study the American people that the typical American, although energetic, resourceful and venturesome, lacks a knowledge of fundamentals. He has the defects as well as the virtues of the pioneer. Are these traits characteristic of the American surgeon? If they are, the fact should be reckoned with in our plans for the training and education of our surgeons. We should foster the energy of the pioneer and give to him the fundamental knowlege needed by a master.

Nowadays a preliminary undergraduate training in the fundamental sciences of anatomy, physiology and pathology is universally recognized as essential for ordinary medical practice. But it is often forgotten that within the necessarily brief time allotted to these laboratory subjects in the medical curriculum, only their most elementary phases can be considered. These may be sufficient for the general practitioner, but are certainly inadequate for the specialist. Just as a general knowledge of the basic sciences is essential to their successful application in general practice, so is a more detailed first-hand knowledge of the underlying sciences equally necessary for intelligent specialization in a narrower clinical field. The logical graduate medical course must therefore repeat the order of sequence well recognized in the undergraduate curriculum: first, the fundamental sciences; second, their clinical application. The wouldbe specialist is prone to put the clinical cart before the laboratory horse, however, just as the shortsighted medical freshman undergraduate would like (and formerly was permitted) to attend clinics from the beginning of the medical course.

For example, let us consider the case of the average medical graduate who desires to become an ophthalmologist. As an undergraduate, he has had opportunity to obtain only the most elementary knowledge of the gross anatomy in the orbital region, possibly supplemented by the dissection of a fresh animal eyeball. His knowledge of the histology and embryology, the neurology and physiology, the pathology and bacteriology, and the pharmacology of the optic apparatus is equally limited. But for the ophthalmologist, a much more detailed and special knowledge of the ocular system from these various standpoints is obviously the first essential in a logical plan of graduate training. The same principle holds for other clinical specialties, the character and extent of the necessary fundamental training in the individual branches varying according to circumstances. While the prospective surgeon usually needs more anatomy and pathology, the pediatrician or the internist as a rule needs rather to concentrate on physiology and chemistry at the beginning of his graduate work.

The principle that clinical medicine is an applied science is thus even more true for specialism than for general practice. Before the wouldbe clinical specialist has acquired a more thorough knowledge of the corresponding fundamentals, he simply does not have the science to apply. Without this science, the resultant training and practice will be empiric rather than scientific. Until this elemental principle is clearly recognized, we cannot hope for a satisfactory solution of the problem of graduate medical education for specialism. "Back to the laboratory" must be the slogan for the wouldbe specialist in the first stage of his graduate training.

Granting the principle, where can the requisite fundamental science be obtained? Practically the only places where the necessary facilities are now available are the laboratories of the medical schools of the stronger universities. Some of the larger hospitals have fairly good laboratories of pathology and bacteriology, but few have any one available who is capable of guiding the candidate in a systematic advanced study, even where there are laboratory facilities. The other laboratories necessary for graduate training are not provided by hospitals.

On account of the great expense involved, it likewise appears utterly impossible for the independent postgraduate medical schools to establish satisfactory laboratories for advanced scientific work. While these schools may serve a useful purpose in functioning as "undergraduate repair shops" (Flexner), their facilities for the adequate training of competent specialists are decidedly limited. If the day has passed when independent undergraduate medical schools can exist on the basis of support from tuition fees, what must we conclude as to graduate medical instruction, which, if properly given, is still more expensive? This matter has been fairly and fully discussed by Flexner, and also by Arnold in the report of the American Medical Association committee to investigate graduate medical instruction. Arnold suggests that the problem would be solved if the independent postgraduate schools could become affiliated with the existing undergraduate medical schools of the various universities.

Even the universities which have adequate laboratory staffs and facilities for this purpose have rarely given much thought to the matter of providing the opportunities necessary for the training of clinical specialists in the fundamental sciences. Careful planning is required to give the best results. The course for each student should be arranged by conference between those in charge of the various clinical specialties and those in charge of the corresponding laboratory subjects involved. The essential point is that each student should have his work well outlined and supervised and that he should be provided with the necessary laboratory material and facilities. On account of the relatively small number of students requiring such work, it must necessarily be chiefly individual in character. Working almost entirely alone, with the occasional conferences and limited aid of the teacher, will encourage independence and resourcefulness on the part of the graduate student. If at this stage he is unable to work things out for himself, given the requisite direction and facilities, he would better abandon his ambition for the higher education for which he is evidently unqualified.

Systematic provision should be made in a larger number of universities for at least this fundamental laboratory training needed by clinical specialists. Even the two-year medical schools, or other schools lacking sufficient clinical facilities for complete graduate training, could without much difficulty organize an excellent year's fundamental course for this purpose. A discussion of the curriculum requirements for graduate training in the various specialties may be found in papers by Todd, Sedgwick, Litzenberg, Edward Jackson, Duane, Shambaugh, Wishart and others. The broader phases of the subject are emphasized by Christian, Barker, Lyon and Wilson.

If the student could also have an opportunity to serve as a teaching assistant in one of the fundamental laboratories during the earlier part of his graduate work, so much the better. The road to specialism through an apprenticeship in one of the basic sciences, long recognized in Europe, has thus a substantial justification. As Melzer has well said:

> The best physician of the future will be the brainy man who has spent many years in studying the methods employed in acquiring knowledge in the pure medical sciences, and then in applying all his mental energies to a broad study of disease.

The clinical graduate student should also profit greatly from his association with the laboratory sciences in ways other than the acquisition of indispensable information. He should also obtain what is really even more important, an inspiration of scientific ideals and a preliminary training in scientific methods. He should furthermore avoid the fallacious idea that he can leave the laboratory behind him when he has finished therein his preliminary training. Rather will he find it necessary, if his clinical career is to be progressive and fruitful, to maintain permanent relations with the basic medical sciences. Unless he catches the scientific spirit, and realizes that all medicine is essentially merely one phase of biology in a broad sense, his sojourn in the laboratory will have been in vain.

CLINICAL TRAINING FOR SPECIALISM

Following the preparatory work in the fundamentals, the clinical training for specialism is naturally of paramount importance. The apprenticeship method by which most specialists in the past have obtained this training in connection with private or public clinics has varied exceedingly in the opportunities offered and the results obtained. At best, while offering excellent practical training, it has usually been deficient in the requisite fundamental laboratory instruction above mentioned. Moreover, it has usually tended to become more or less routine in character, particularly when there is a large volume of clinical work to be done, with an inadequate number of assistants.

The objections to overloading the student with routine clinical work (during the internship) are thus emphasized by Dodson:

> The amount of routine work now demanded of the intern in many hospitals so consumes his time as to make it very difficult and often impossible for any but the exceptional student to prepare a thesis. This ought not, however, so to be. The intern should have time to study the cases assigned to him thoroughly and carefully, and also pursue exhaustively the study of some disease or condition as presented by some interesting case, or group of cases, to explore the literature relating to that topic, and to set down in orderly fashion the results of his investigation. No instruction or experience in his career is so potent an educational measure as this one of independent creative work.

The argument naturally applies even more strongly in the cases of graduate students beyond the internship year who are preparing themselves to qualify as specialists.

Even in our own university work, where the educational principles are especially emphasized, the graduate fellows in clinical departments have sometimes found that the large amount of routine work they are required to perform leaves insufficient time and energy for the study necessary to meet the requirements of the graduate school. It does not appear possible for the graduate student to study as he should, if he has, free from routine service, only one or two half-days a week, supplemented by holidays, nights and Sundays! The war has, of course, made this question more acute, on account of the universal shortage of medical assistants in the various clinics; but now that the emergency is over, it should be possible to avoid this difficulty by providing a larger staff of clinical assistants. In my opinion, the ideal educational results are not possible unless the graduate student has at least half of his time free from routine clinical service. Our experience has shown that it is otherwise difficult for those holding graduate fellowships to fulfil the requirements in scholarship for the higher (doctorate) degree within the usual three-year period.

While on the one hand a thorough, practical training in the clinical work is indispensable, it is clearly evident that this phase of the work may easily be overemphasized, leading to a neglect of the scholarship requirements.

REQUIREMENTS IN SCHOLARSHIP

The question may fairly be raised as to whether these scholarship requirements are really necessary for those seeking to qualify for leadership in clinical specialism. Or are these requirements merely "academic frills" of no real value? The requirements are based on the theory that technical proficiency alone is insufficient to train the highest type of physician; that practical experience must be supplemented by theoretical study; that the ideal of skill must be accompanied by the ideal of scholarship, if we are to train clinical specialists of the highest type, who can render the greatest service to mankind, in research as well as in practice. Let us see whether there is any adequate basis for these assumptions.

In academic circles, technical skill is generally rated somewhat low as an educational factor. On the other hand, by professional men scholarship is frequently scorned as "impractical" or "theoretical." But as Allbutt maintains:

> Surely by abstraction, or theory, we mean the rules by which present or concrete problems are most rapidly and economically solved; so that he who has the largest possessions in theory will solve these problems most quickly and efficiently. A theory, or abstraction, is indeed a summation of the experience of the race on which the future must be built.

Minot puts it thus:

> One still sometimes hears the distinction drawn between the "practical" and the "scientific" doctor; so far as I know, the most important difference between a "practical" doctor and the truly scientific is that the patients of the former are more likely to die.

Or as summarized by Keefe:

> The cunning of the hand is but the outward and visible sign of the power of the brain which directs it, and the surgeon who would be a good craftsman must first be a good thoughtsman.

From the broader point of view, considering education as the sum total of all factors which train man for better adjustment with his environment, both skill and scholarship are essential. Activity without intelligence is as fruitless as intelligence without activity. Knowing and doing must go hand in hand, if education is to be efficient. This principle is now generally recognized in undergraduate medical education. Is not the power of independent thought and productive scholarship even more important in graduate medical education? On this principle are based the graduate school requirements of thesis, foreign language and history of medicine. Let us briefly consider each of these in turn. The thesis involves a thorough training in scientific research methods, and the application of these methods in the solution of some medical problem. The emphasis on this phase of graduate work, this training in productive scholarship, seems to me justified in two ways.

In the first place, we all recognize that, in spite of great advances in the past, medical science is only at the threshold of its future possibilities. Suffering humanity cannot wait for medical discoveries to come by chance. The state should therefore encourage and train research workers in every branch of clinical medicine, as well as in the more fundamental sciences. This is, I believe, the highest function of the university graduate school. Although only a few of the graduate students will actually develop into investigators of the first rank, even these few will fully justify all the effort and expense involved in their education. While the skilled specialist in his practice may relieve hundreds, his research resulting in even a comparatively small advancement in his subject will, through the entire medical profession, reach and benefit thousands and millions of human beings.

In the second place, training in rescarch methods, as involved in the preparation of a creditable thesis, is also exceedingly valuable even for the larger number who will never become primarily researchers (in the narrower sense), but who will rather devote themselves to specialized clinical practice.

As Allbutt reminds us:

> We are prone to forget that research has two purposes, results being but one of them; the other purpose is method, which is itself an education in flexibility, ingenuity, dexterity and perseverance.

Even the "purely clinical" specialist will thus profit greatly by a thorough training in scientific research methods. This training in research methods is directly beneficial to the clinical practitioner because the practice of medicine is, or should be, essentially scientific research. As Barker expresses it:

> Each new patient is a problem for investigation; the clinician applies the methods he has learned and tries to solve it.
>
> The essentially inquisitive nature of the everyday work of the clinician has to be personally known to be fully appreciated. In addition to the ordinary routine of clinical work, physicians are constantly trying out new methods of examination and treatment that have been devised and recommended; this work, carefully done, the observations being accurately recorded and analyzed, affords material for statistical inquiry, the results of which are of high importance to the profession. When a new method of determining clinically the size of the heart has been proposed, or an early diagnostic test for typhoid fever or for syphilis or for carcinoma of the stomach suggested, long experiential proving in the clinics must decide as to its value before it is accepted for general routine. And therefore the students "should be encouraged to apply the methods of modern scientific investigation to the objects which they, themselves, observe. *Thus introduced to the spirit and method of scientific inquiry, the students are protected from the dogmatism which so often accompanies mere traditional teaching.*"

The importance of the research spirit in medical education has likewise been emphasized by Pearce and others. In its broader aspects, this subject is receiving increasing recognition as a principle of education in general, and may, let us hope, be extended throughout our entire educational system, from the kindergarten to the university. From this broader point of view, Donaldson's conception of the research spirit may be cited:

Research is a frame of mind. A man may have little leisure and trifling resources, may never have published, but if he examines his world in a questioning spirit, if he carries with him not only conclusions, but the observations on which they rest, if he refuses to pound square facts into the round holes that he happens to have in hand, he has attained illumination. The spirit of research is nothing mysterious or remote; it is everyday hard sense. Unfortunately, that does not make it common, but it does make it attainable.

I have dwelt thus somewhat at length on the research spirit, because if it is so important to medicine in general, it should clearly be a very essential part of the highest phase of medical education, the graduate training of specialists. And the thesis is merely the concrete evidence that the student has caught the research spirit, that he has had adequate training in research methods and in their successful application in the solution of some definite medical problem.

In order to indicate more clearly the types of problems which may be utilized for this purpose, the following list of thesis subjects may be of interest. It includes those for the higher degrees awarded in the clinical branches by the Graduate School of the University of Minnesota during the past two years (1917 and 1918).

G. L. McWhorter, Ph.D. (Surgery): "Some Clinical and Experimental Observations on Gastric Acidity."

Rood Taylor, Ph.D. (Pediatrics): "Hunger in Infants."

H. W. Woltmann, Ph.D. (Neurology): "The Brain Changes Associated with Pernicious Anemia."

R. E. Morris, Ph.D. (Medicine): "The Graphic Recording of Reflexes, Clonus and Tremors."

E. L. Crispin, M.S. (Medicine): "Clinical Studies in Abdominal Disorders."

F. B. McMahon, M.S. (Surgery): "Primary Pulmonary Carcinoma and Its Roentgen Diagnosis."

H. W. Meyerding, M.S. (Orthopedic Surgery): "Cystic and Fibrocystic Disease of the Long Bones."

A. W. Adson, M.S. (Surgery): "Experimental and Clinical Results of Nerve Anastomoses."

V. C. Hunt, M.S. (Surgery): "Torsion of Appendices epiploicae."

F. A. Olson, M.S. (Surgery): "A Study of the Roentgenographic Findings in Renal Tuberculosis."

J. de J. Pemberton, M.S. (Surgery): "Blood Transfusion."

These represent scientific studies fulfilling the requirements of productive scholarship on topics which are also of obvious importance in the practical phases of clinical medicine.

As a corollary to the thesis requirement comes the necessity for a study of the medical literature. This involves a reading knowledge of the principal foreign languages. One foreign language is already an admission requirement to the undergraduate work in medical schools of the better class. The addition of a reading knowledge in the second foreign language is not a burdensome requirement, and is an essential prerequisite for efficient scholarship. It is evident that one cannot well invade the unknown until he has learned what is already known in that particular phase of the subject. To do this, he should be able to read at least the three modern languages (English, French and German) in which the greater part of the original work in medical science is published. Translations are usually inadequate and frequently unavailable, even in abstract.

An appalling amount of time and energy is therefore unnecessarily lost by investigators who neglect the literature of their subject. As Cannon has expressed it:

> Research implies in the first place *seeking again* over a region which has been previously traversed in order to learn what other men have done and the point where their labors ended. To make progress sure, therefore, previous records must be carefully studied. The failure to pay this just tribute to those who have labored before us has not seldom led to fruitless effort or to vain repetition of work already well done. Marking the boundaries demands, then, a scholarly acquaintance with the earlier discoveries; and the painstaking methods of the scholar must be used.

Even the genius of John Hunter did not prevent him from serious error (as cited by Jacobi) in denying the occurrence of visceral syphilis, which had already been demonstrated by Morgagni and Paracelsus. Numerous similar instances could be cited in every branch of medicine, indicating the necessity of a reading knowledge of foreign languages for efficient medical research. A knowledge of languages is necessary even for practitioners who desire merely to keep abreast of medical progress; and it is indispensable for those who aspire to leadership.

It should also be insisted that since the foreign language is required as a tool in productive scholarship, it must be a practical reading knowledge. It is not sufficient merely to have had at some time in the past one, two or more years of formal language study. The essential requisite is the present *ability to use* the language effectively in reading the literature of the major subject, which is often quite a different matter. The only way to insure this knowledge is by a practical test, such as that required by the graduate school.

Associated with the thesis and language requirements is the subject of the history of medicine. Candidates for the higher degrees in medicine at Minnesota are required to know the history of medicine in general, and particularly that of the major subject in which the degree is sought. This requirement is based on the necessity for a background of historical knowledge, without which it is impossible to get an adequate conception of present conditions. The history of medicine teaches the evolutionary stages by which progress has been made in the past, and reveals pitfalls which must be avoided in the future. Probably in no department of human activity is a knowledge of its historical development more important than in medicine. The neglect of this subject in the undergraduate medical curriculum merely serves to emphasize the necessity for its requirement in the graduate work.

CERTIFICATION

It is not sufficient merely to provide adequate training for medical specialists. If they are to serve the public, some means must be devised to certify their proficiency. Realizing this necessity, the American Board for Ophthalmic Examinations (representing the national ophthalmic societies) examines those who have had the minimum training required for specialists in ophthalmology and awards certificates to those successful in passing the examination. Similarly membership in the American College of Surgeons indicates the recognition of ability in the practice of surgery.

While certificates and titles of this character will undoubtedly be useful in helping to designate those at least moderately well qualified to practice in their special fields, it would seem that for those who have devoted a period of three years or more to a systematic course of graduate training and study a university degree is a more appropriate form of recognition and certification. No other institutions are so well qualified as the university to set standards which must command respect.

Just what this degree should be is a matter which has been much discussed, and concerning which there is still no unanimity of opinion. In the University of Colorado School of Medicine, where graduate work in ophthalmology has been developed through the pioneer efforts of Edward Jackson, the degree of Doctor of Ophthalmology has been awarded to several candidates since 1913. Arnold proposes the degrees of Master of Science in Medicine (M.S.Med.) and Doctor of Science in Medicine (D.S.Med.), which in scholarship are essentially equivalent to the well known graduate degrees, Master of Arts (A.M.) and Doctor of Philosophy (Ph.D.). In addition, he proposes a degree of Doctor of the Practice of Medicine (D.P.Med.). "The requirements for this degree should be essentially the same as for the D.S.Med., except that the time devoted to research and to the preparation of a thesis would be devoted to the development of higher technic and skill in practice."

The latter proposal, to establish a practitioner's degree without research or thesis, is open to serious objections. It would tend to sacrifice scholarship in favor of skill, and thus to yield an unbalanced and undesirable type of specialism. Rather would it seem better to insist that no specialistic training without scholarship requirements involving at least some original work should be crowned by a university graduate degree.

Judging from our experience at Minnesota, graduate students in clinical branches will fall into three groups: Some will be unable to meet the thesis and associated requirements. This deficiency will debar them from being candidates for the higher degrees, irrespective of their technical skill in routine clinical work. Others will be able to produce a fairly creditable thesis, exhibiting some capacity for independent thought, though distinctly below the standard of scholarship ordinarily required for the doctor's degree in the graduate school. These may properly be awarded the degree of Master of Science (M.S.) in the special field. The third class, who measure fully up to the highest standards of both skill and scholarship, are awarded the degree of Doctor of Philosophy (Ph.D.) in the special field involved. We formerly awarded the degree of Doctor of Science (D.Sc.) for the same purpose, but abandoned it in recognition of the growing tendency to use this for an honorary degree.

The use of the M.S. and Ph.D. degrees (qualified or unqualified) for graduate work in the medical sciences is in accordance with the recommendations of the Committee on Degrees (A. C. Eycleshymer, chairman) at the recent meeting of the Association of American Medical Colleges in Chicago in March, 1919. The Ph.D. degree, as pointed out by Shambaugh, Vincent, Lyon and others, has the advantage of being thoroughly established, carrying with it everywhere the certification of ability in original thought and training in scientific methods. The qualification of the degree by the addition of the special field of clinical medicine involved should add to this a further recognition of practical ability in that professional field. It should indicate to the world that the recipient of this degree has undergone a long and careful training, both theoretical and practical; that he has met the most rigid tests both of skill and of scholarship; and that he is well qualified for leadership in his chosen field of specialism in medicine.

CONCLUSIONS

We must recognize an increasing need for medical specialism, especially in connection with the development of the group system in medical practice. For the training of efficient specialists, adequate facilities are in general available only in the medical schools of the stronger universities. These schools should organize graduate work for systematic training of medical specialists along broad lines, including the necessary foundation in fundamental scientific work, practical clinical instruction and training in research methods. Work thus planned in accordance with the ideals of skill and scholarship will yield the most efficient type of specialist. Successful candidates may approximately receive the degree of Master of Science or Doctor of Philosophy, specifying the field of proficiency.

REFERENCES

Allbutt, T. C.: On Professional Education with Special Reference to Medicine, London, The Macmillan Company, 1906.

Arnold, H. D.: The Problems of Graduate Medical Instruction, J. A. M. A. **64:** 329-332 (Jan. 23) 1915.

Report of the Committee to Investigate Graduate Medical Education, Eleventh Annual Conference of the Council on Medical Education, A. M. A. Bull. **10:** 298-307 (March) 1915.

The Problem of Higher Degrees in Medicine, Thirteenth Annual Conference of the Council on Medical Education, A. M. A. Bull. **12:** 207-212 (March) 1917.

Barker, L. F.: Some Tendencies in Medical Education in the United States, J. A. M. A. **57:** 613-621 (Aug. 19) 1911; reprinted in Medical Education and Research, 1913.

Bispham, W. N.: Discussion on Medical Education, Fifteenth Annual Conference of the Council on Medical Education of the A. M. A., J. A. M. A. **72:** 890 (March 22) 1919.

Bradford, E. H.: The Higher Education in Surgery, Denver M. Times **34:** 315-320, 1914-1915; abstract in American College of Surgeons, a List of Fellows, Chicago, 1914.

Cannon, W. B.: The Career of the Investigator, Science (July 21) 1911; reprinted in Medical Education and Research, 1913.

Christian, H. A.: A Career in Medicine and Present-Day Preparation for It, Science (Oct. 22) 1909.

Dodson, J. M.: The Addition of a Fifth Year to the Medical Curriculum, J. A. M. A. **59:** 589-593 (Aug. 24) 1912; reprinted in Medical Education and Research, 1913.

Dodson, J. M.: Problems in Medical Education, J. A. M. A. **72:** 821-822 (March 15) 1919.

Donaldson, H. H.: Research Foundations in their Relation to Medicine, Science (July 19) 1912; reprinted in Medical Education and Research, 1913.

Duane, A.: The Ideal Medical Education for the General Practitioner and the Specialist. N. Y. State J. M. **11:** 104-111, 1911.

Flexner, A.: Medical Education in the United States and Canada; A Report to the Carnegie Foundation for the Advancement of Teaching, Bull. No. 4, New York, 1910.

Garrison, F. H.: An Introduction to the History of Medicine, 2nd Ed. Saunders Co., Philadelphia and London, 1917.

Jackson, Edward: The Best Time to Prepare for Special Practice, Amer. Acad. Opth. and Oto-Laryngol., 1913, p. 295.

Report of Committee on Education in Ophthalmology, Tr. Sect. Ophth. A. M. A., 1914, p. 395, and 1915, p. 230.

Jacobi, A.: Some of the Benefits Derived from Medical Libraries, Cleveland M. J. (Nov.) 1909.

Keefe, J. W.: President's Address, Trans. Am. A. Obst. and Gynec. **30:** 1-7, 1917, York, Pa., 1918.

Litzenberg, J. C.: The Graduate Degree in Obstetrics and Gynecology, Am. J. Obst., N. Y. **78:** 404-409, 1918.

Lyon, E. P.: Graduate Education in the Clinical Branches, and the Minnesota Experiment, J. A. M. A. **69:** 1307-13 (Oct. 20) 1917.

Meltzer, S. J.: The Aims of the Association of American Physicians, President's Address, Tr. A. Am. Phys. Thirtieth Session, Washington, D. C. **30:** Philadelphia, 1915.

Minot, C. S.: Certain Ideals of Medical Education, J. A. M. A. **53:** 502-508 (Aug. 14) 1909; reprinted in Medical Education and Research 1913.

Munson, E. L.: The Needs of Medical Education as Revealed by the War, J. A. M. A. **72:** 822-823; 1050-1055 (March 15) 1919.

Pearce, R. M.: Medical Research and Education, Science Press, N. Y., and Garrison, 1913.

Reber, Wendell: The Future of Postgraduate Ophthalmic Education in America, Am. Med., N. S. **10:** 595-606 (Aug.) 1915.

Sedgwick, J. P.: The Requirements for the Higher Degree in Pediatrics, Trans. Assoc. Amer. Teachers of Diseases of Children, Eleventh Annual Meeting, New York City, June 4, 1917, pp. 12-20.

Shambaugh, G. E.: The Preparation of the Specialist; a Problem in Medical Education J. A. M. A. **49:** 540-543 (Aug. 17) 1907.

Todd, F. C.: Higher Preparation in Ophthalmology and Oto-Laryngology, Eleventh Annual Conference of the Council on Medical Education, A. M. A. Bull. **10:** No. 4, 288-295 (March 15) 1915. Also in Ophthalmology (Seattle) **11:** 659-669, 1915.

Vincent, George E.: The University and Higher Degrees in Medicine, J. A. M. A. **64:** 790-794 (March 6) 1915.

Warner, A. R.: Hospital Standardization, with Especial Reference to Educational Work in Hospitals, J. A. M. A. **72:** 914-916 (March 29) 1919.

Welch, W. H.: The Relation of the Hospital to Medical Education and Research, J. A. M. A. **49:** 531-555 (Aug. 17) 1907; reprinted in Medical Research and Education, Science Press, N. Y., and Garrison, 1913.

Wilson, L. B.: The Status of the Graduate Degree in Medicine, Science **46:** 127-131, 1917.

Graduate Work in Medicine in the University of Minnesota, Journal-Lancet **38:** 1-5, 1918.

Wishart, J. S.; Richardson, C. W., and Smith, C. M.: Teaching of Oto-Laryngology. Report of the Committee Appointed to Consider the Best Methods to be Followed in the Teaching of Oto-Laryngology in Undergraduate and Post-Graduate Schools, J. A. M. A. **61:** 535-537 (Aug. 23) 1913. Cf. also the symposium by Mygind and others in the Brit. M. J. Aug. 24, 1912.

Reprinted from The Journal of the American Medical Association
Aug. 2, 1919, Vol. 73, pp. 307-311

Copyright, 1919
American Medical Association, 535 N. Dearborn St., Chicago

9h

[Reprinted from MINNESOTA MEDICINE, Nov. 1918]

THE EFFECTS OF UNDERFEEDING AND REFEEDING UPON THE GROWTH OF THE VARIOUS SYSTEMS AND ORGANS OF THE BODY.*

C. M. Jackson, M. S., M. D., and
C. A. Stewart, Ph. D.
Institute of Anatomy, University of Minnesota.
Minneapolis, Minn.

A few years ago, Waters ('08) made a remarkable discovery. He found that calves stunted by underfeeding continue to grow in height and width, even when the body weight fails to increase. Thus under these conditions the skeleton persists in growth at the expense of the remainder of the body, which becomes greatly emaciated. Aron ('10, '11) confirmed this result in dogs, finding that in puppies underfed for several months the skeleton, and apparently also the brain, continue to grow at the expense of the rest of the body. The loss was found to fall chiefly upon the fat and muscle, the viscera apparently undergoing but little change in weight. A few clinical observations by Variot ('07, '08), Freund ('09), Birk ('11), and Hess ('16) show a persistent tendency to increase in the length of the body in malnourished infants. This would indicate that the skeleton in young children likewise may continue to grow under conditions of chronic underfeeding or malnutrition with retarded or stationary body weight.

An extensive investigation of this matter was therefore undertaken in the Department of Anatomy at the University of Minnesota in order to determine: (1) what changes take place in the various organs and parts of the entire body as the result of underfeeding; (2) whether the effects are different at different ages or with different degrees of inanition; (3) how the results on young individuals compare with those in adults subjected to acute or chronic inanition; and (4) how various periods of underfeeding the young, may affect their growth later when they are fully fed.

---

*Presented before the Annual Meeting of the Minnesota State Medical Association, held at Duluth, Minn., Aug. 29 and 30, 1918.

I. Effects of Underfeeding upon Growth in the Young.

The white (albino) rat was selected as the most convenient form for the experiments. It breeds rapidly and thrives in captivity. Its small size and rapid growth (reaching sexual maturity at 10 weeks, and adult size within a year) make it possible to obtain extensive data in a comparatively short time. The normal growth and variability of the rat and its various organs have also been worked out very thoroughly (chiefly by Donaldson, Hatai, Jackson and Lowrey), and normal growth tables by Donaldson ('15) are available for comparison. This is a matter of great importance, for the lack of knowledge concerning the normal structure and variability of the animal used frequently makes it very difficult to draw trustworthy conclusions from the results of experimental work.

The results of our investigations showing the relative changes in the weights of the component systems and organs are summarized in the accompanying table. The data are based upon the observations of Jackson ('15, '15a) and Stewart ('18). The relative changes in weight are indicated in percentages of increase (+), or decrease (—), in the weight of the organ or part in comparison with its weight in a normal animal of the same body weight. For the adult rats with acute or chronic inanition (last two columns), the normal organ weight at the initial

Average Percentage Changes in the Weight of Systems and Organs in Albino Rats Underfed at Different Ages. Younger Nornal Controls of Same Body Weight Used for Comparison, Excepting the Adult Rats, Where Controls of Same Initial Body Weight Were Used.

	Young Rats						Adult Rats	
Organs	Held at birth wt. av. 16 da. Body wt. 5 g. (Stewart '18b)	Underfed from birth to 3 wks. Body wt. 10 g. (Stewart '18)	Underfed from birth to 10 wks. Body wt. 15 g. (Stewart '18)	Underfed from age 3 to 10 wks. Body wt. 24 g. (Jackson '15a)	Underfed from age 3 wks. to 1 yr. Body wt. 52 g. (Stewart '18)	Underfed from 10 wks. to 8 mo. Body wt. 80 g. (Jackson '15a)	Acute Inanition Body loss 33% (Jackson '15)	Chronic Inanition Body loss 36% (Jackson '15
				A. Systems.				
Visceral Group	+ 46%	+ 28%	+ 38%	+ 1%	+ 1%	— 21%	— 40%	— 36%
Integument	+ 25	+ 0	— 48	— 36	— 11	— 16	— 31	— 39
Skeleton	+ 6	+ 19	+ 24	+ 28	+ 32	+ 0.2	— 0.4	+ 2
Musculature		+ 8	+ 10	+ 3	+ 4	+ 25	— 31	— 41
Remainder	— 59	— 40	— 23	— 2	— 33	— 5	— 28	— 44
				B. Organs.				
Testes	+374	+188	+ 51	+ 34	— 42	+ 2	— 30	— 40
Epididymis	+225	+ 95	— 6	—	— 32	—	—	—
Eyeballs	+146	+ 41	+ 66	+ 50	+ 73	+ 54	— 4	— 6
Brain	+125	+ 60	+ 8	— 0.5	+ 4	+ 0	— 5	— 7
Spinal Cord	+ 83	+ 70	+ 70	+ 36	+ 40	+ 36	— 0	— 4
Kidneys	+ 90	+ 21	+ 38	+ 4	— 6	— 7	— 26	— 27
Stomach-Intest	+ 40	+ 17	+100	+ 28	— 27	— 26	— 57	— 57
Spleen	+ 38	— 49	+ 24	— 42	— 5	— 1	— 51	— 29
Hypophysis	+ 29	+ 33	+ 24	+ 18	— 3	— 6	— 26	— 25
Heart	+ 26	— 5	+ 27	— 1	— 10	— 6	— 28	— 33
Ovaries	+ 5	+ 83	+ 54	— 27	+ 17	— 54	+ 22	—
Suprarenals	+ 5	+ 60	+114	+ 26	+ 48	— 26	+ 2	— 9
Thyroid	+ 8	+ 5	+ 4	— 24	— 36	— 62	— 0	— 22
Lungs	+ 3	— 26	— 26	— 15	+ 28	— 13	— 31	— 40
Liver	— 23	+ 17	+ 64	+ 10	— 7	— 39	— 58	— 43
Thymus	— 49	— 30	— 80	— 90	— 90	— 90	—	—

body weight, at the beginning of the experiment, is used as a basis for comparison. So far as possible, normal controls were taken from the same litters as the test rats.

The first column in the table shows the average changes in a series of 15 rats (8 males, 7 females) held at birth weight (about 5 grams) by underfeding up to 11 to 22 days of age, the average being 16 days. The rats were retarded in growth during this period by removing them from the mother for various successive periods to prevent nursing. Normally during this period the rats would more than treble their body weight, so the repression of growth is very severe. In the human species, it might be compared with an underfeeding sufficient to hold the newborn infant at birth weight for about a year (the time normally required to treble the birth weight).

The second column indicates the relative changes in a series of 7 rats (4 males, 3 females) similarly underfed from birth to 3 weeks of age. In this case, however, the underfeeding, though extending over a slightly longer period, was somewhat less severe. The rats were allowed to increase slowly in body weight from about 5 grams to 10 grams. Normally they would reach 20 to 25 grams in the same period. Another series of rats (not given in the table) was underfed from birth to 6 weeks, the body weight increasing to about 13 grams. The results were in most cases intermediate between those of the second and third columns.

The third column indicates the changes in a series of fourteen rats (seven males, seven females) in which the underfeeding extended from birth to 10 weeks of age. After the weaning period (age of 3 weeks) the young were fed restricted amounts of bread and milk. They slowly increased to 15 grams in body weight, the normal at 10 weeks being more than 100 grams. In the human species, this would be roughly comparable to a child about 12 years of age stunted by underfeeding from birth so as not to exceed the normal weight of an infant at 1 year of age, or about 20 pounds.

The fourth column indicates the average results in a series of 19 rats (8 males, 11 females) in which the experiment did not begin until the rats were weaned at 3 weeks of age, with an average body weight of about 24 grams. They were then held at constant body weight by underfeeding up to the age of 10 weeks.

The fifth column indicates the results in a series of 4 rats (2 males, 2 females) in which the underfeeding likewise began at 3 weeks, but extended over a much longer period, more than a year. It was impossible to maintain the rats alive at constant body weight for so long a period, the minimum of 52 grams representing an increase to about double that when the experiment began at 3 weeks. In human terms, this would correspond roughly to an adult dwarfed by underfeeding from infancy so that the body weight would not exceed that of a five year old child.

Another series of 6 rats (4 males, 2 females) were underfed from 3 weeks to about 10 months, permitting the weight to increase slightly more, the average final weight about 77 grams. The results (not included in the table) were in most cases similar and intermediate between those of the fifth and sixth columns.

The sixth column indicates the results in a series of 4 rats (3 males, 1 female) underfed at a later period, beginning at the age of 10 weeks and held at nearly constant body weight up to the age of about 8 months, the final body weight averging about 80 grams. This in the human species would correspond to a period extending from the age of puberty nearly to adult age. For this group of rats direct controls were not available. They were compared with Donaldson's norms for the same body weight.

The last two columns indicate the change in weight of the various systems and organs in adults rats subjected to inanition. The acute inanition group included 15 rats (11 males, 4 females) averaging about 250 grams initial body weight. They were given water only for 6 to 12 (average 9) days, and were killed and autopsied with loss in body weight ranging from 25 to 39 (average 33) per cent. The chronic inanition group (6 males) were fed gradually decreasing amounts, so as to lose an average of about 36 per cent in body weight in about 5 weeks. As previously stated, the changes during adult inanition are reckoned on a basis somewhat different from that used for the younger rats, but the results are interesting for comparison.

We may now consider the individual systems and organs at various ages and under different degrees of inanition as shown in the table. The first part of the table ("A. Systems") gives the component systems: visceral group, integument, skeleton, musculature and "remainder"; while the second part ("B. Organs") includes the various organs of the visceral group.

Skeleton.—First, it may be noted that the data for the skeleton in every case indicate a persistent growth for this system in the stunted young rats. Their body length, and especially their tail length, in all cases markedly exceeds that in the normal controls of the same body weight. The figures given in the table are for the weight of the entire ligamentous skeleton, but the cartilaginous skeleton shows a similar increase. The dried skeleton shows a much greater relative increase, indicating that the solids are increasing relatively, as happens in the normal development of the bones. That the persistent growth of the skeleton in the undernourished body in these cases involves the normal process of ossification is likewise indicated by the appearance and union of various centers of ossification. The cartilage of the external ear shares in the persistent growth and the third molar teeth develop during the period while the increase in body weight is inhibited by underfeeding.

In the young rats held at birth weight, the skeleton and musculature were not separated. Their combined weight, however, indicates an average increase of but 6 per cent, which is much smaller than that for the succeeding periods. This suggests that during severe inanition immediately after birth the skeleton growth may be inhibited to a greater extent than later. This is rather surprising, since the normal growth rate of the skeleton is relatively greatest in the first week after birth (Jackson and Lowrey '12). The marked inhibition of skeletal growth in the first group may be due to the greater severity of the underfeeding, however, as in the second group with a less restricted growth over a slightly longer period the skeletal increase (average 19 per cent) is much greater. Freund ('09) and Birk ('11) likewise noted that malnutrition in the human infant retards growth in body length more in the newborn than at later periods. The data in the table also indicate that the skeletal growth is feebler (practically at a standstill) in the rats underfed at a late period, from 10 weeks to 8 months of age. This is perhaps because the skeleton has in this case largely completed its growth before the inanition began. At any rate the conclusion of Waters ('08) and Aron ('10 '11) that the growth impulse during underfeeding is strongest in the skeleton apparently does not apply to the earliest postnatal periods, in which the growth is stronger in the integument and visceral group, nor to the later, adolescent period, where it appears stronger in the musculature. In adult rats, the skeleton remains nearly constant in weight during inanition, as shown in the table.

In general, the results are of clinical interest because they indicate that the growth impulse of the skeleton is especially strong during infancy and childhood, and tends to persist even in conditions of chronic malnutrition which prevent or greatly retard increase in body weight. Whether the same rule applies in conditions other than simple inanition, we do not know. It would in any event appear to be very desirable to have clinical records kept of the body length as well as of the body weight under such conditions. Cases in which the length of children remains stationary for any considerable period show a much more profound disturbance of the fundamental processes of growth, and should usually be considered much more serious than those in which merely the body weight is involved.

Musculature.—The changes in the weight of the musculature differ from those of the skeleton in showing generally but a slight increase during the various inanition periods. The conditions are reversed, however, in the later period of underfeeding beginning at 10 weeks. Here there appears a marked increase (average 25 per cent) in the weight of the musculature, although the skeleton apparently remains nearly stationary. Here the difference may depend upon the normal growth tendency, which at this period is much stronger in the musculature than in the skeleton (Jackson and Lowrey '12). In the adult, both acute and (especially) chronic inanition produce marked loss in the weight of the musculature, in which the loss is roughly proportional to that in the weight of the entire body.

Integument.—The integument differs from the skeleton and musculature in that it generally shows a marked loss in weight in the young rats during underfeeding. The loss appears relatively greatest, averaging 48 and 36 per cent, in the two middle groups. At the later periods the loss is relatively smaller. In the group underfed from birth to 3 weeks, however, the integument remained about stationary, while in the first group, held at birth weight, the integument gained an average of 25 per cent in weight. As shown by Jackson and Lowrey, the normal rate of growth for the integument is relatively greatest during the first week after birth, which perhaps may explain its strong tendency to grow at this early period even under very unfavorable nutritive conditions. The loss in the weight of the integument during adult inanition is marked, and nearly proportional to that of the body weight. In this case, and in the later periods for the young rats, the loss in weight of the integument is doubtless largely due to loss of fat. The loss in the integument and musculature throws the more resistant skeleton into great prominence, giving the characteristic picture of emaciation of the body.

Visceral Group.—The visceral group as a whole varies remarkably in growth tendency according to the age at which the underfeeding period begins. The growth tendency appears strongest in the three younger groups, in which the experiments began at birth. It is most striking in those held at birth weight for an average of 16 days, the visceral group meanwhile increasing 46 per cent in weight. In the other two younger groups, the average increase is 28 and 38 per cent.

In the next two groups, the underfeeding beginning at 3 weeks of age, the visceral group has apparently lost its earlier growth impulse, and is now barely able to hold its own weight. The results at these two periods therefore agree with those of Aron ('11) in puppies stunted by underfeeding. In the rats beginning the experiment later at 10 weeks, however, there is a definite loss (average 21 per cent) in the visceral group. Thus as age advances the conditions tend to approach those of the adult rat, in which there is in the visceral group during acute or chronic inanition a marked loss in weight, roughly proportional to that in the weight of the entire body. As will be seen later, however, the individual viscera are by no means uniform in their weight changes during inanition, the present result being merely the average for the whole group and being determined principally by the weight of the larger organs, especially of the liver.

Remainder.—The "remainder" in the table is the difference between the net body weight (excluding intestinal contents) and the combined weight of the skeleton, musculature, integument and viscera. It includes, in addition to loss by evaporation, certain small unweighed organs, together with the interstitial tissues and fluids, and the blood escaping from the organs at autopsy. This "remainder" is somewhat variable in amount, but is relatively largest in the new born (about 21 per cent of the body weight, according to Jackson and Lowrey), which is very rich in interstitial fluids. From one week of age the "remainder" normally averages 12 or 13 per cent of the body. In the table it will be noted that the "remainder'' always decreases during inanition, although the amount varies at the different periods. The loss appears greatest (average 59 per cent) in the first group, held at birth weight, decreasing in the succeeding groups. In adult inanition the loss appears much greater in chronic inanition (44 per cent) than in acute inanition (28 per cent), due perhaps to a greater loss of fat in the former case.

Where the body weight remains constant, or where controls of the same final body weight are taken, it is evident that the increase in some parts should be offset by a corresponding decrease in others. Thus the gains in the visceral group, integument, skeleton and musculature in the first two series are counterbalanced by loss in the ''remainder.'' (Some apparent discrepancies in the results are due to experimental error, individual variations and lack of corrections for differences in body weights between test rats and controls.) At later stages, the gains in the skeleton and musculature are offset by losses in the integument and ''remainder'' and (in the last series) in the viscera. In the skeleton, the visceral group, and, to a certain extent, the integument, the conditions in the losses of the various systems in young rats at successive ages tend progressively to approach those found in adult rats with acute or chronic inanition. This rule apparently does not apply to the musculature or the ''remainder,'' however. In addition to the changes in growth reactions during underfeeding at different ages, there are evidently marked differences according to the severity of the underfeeding. This is evident by a comparison of the results in the first two columns, in which the age was nearly the same, but the extent of the repression in body weight quite different.

Likewise in adults the results differ somewhat in acute and in chronic inanition.

The Individual Organs.—As shown by part "B" of the table, the individual organs of the visceral group vary to an extraordinary degree in their growth reactions during the underfeeding experiments. This applies not only to the various individual organs, but also in most cases to the same organ at different age periods and in different degrees of inanition. In the table, the organs are arranged in decreasing order of their growth tendencies in the first group, held at birth weight.

The **testes** show a most remarkable growth capacity in the earlier experiments. While the body is held at birth weight by underfeeding for 16 days, the testes gain an average of 374 per cent in weight. This extraordinary growth rate is less marked in the later periods, the gains being succesively 188 per cent, 51 per cent and 34 per cent. In the long underfeeding experiment, extending from three weeks to a year of age, the testes decrease 42 per cent in weight, while in the group underfed beginning at ten weeks there is little change in their weight. In adult inanition, the testes suffer a marked loss in weight, roughly proportional to that of the entire body. The changes in the weight of the **epididymis** appear **in general** similar to those of the testes, though not so great. The data for the epididymis are less complete, however.

The **eyeballs** also exhibit a remarkable tenacity of growth during the underfeeding experiments. Although the increase of 146 per cent during the first period (maintenance at birth weight) is somewhat less than that of the testes and epididymis, the growth impulse is more persistent and continues in marked degree. The increase throughout the various subsequent periods varies from 41 to 73 per cent. During adult inanition the eyeballs, like the skeleton, are very resistant, losing only 4 to 6 per cent in weight.

The **spinal cord** exhibits during the various periods of underfeeding a persistance of growth which is very similar to that of the eyeballs, though the increase is relatively less at most periods. The **brain** likewise has a very marked increase (125 and 60 per cent) in the first two groups, but its growth impulse rapidly declines so that it remains nearly constant in weight during the succeeding experimental periods. Stewart ('18a) has further shown that in the persistent growth of the brain in the very young underfed rats, the various parts tend to assume their normal weights, so the brain segments remain normally proportioned. Both the brain and spinal cord are also very resistant toward adult inanition, the loss in no case exceeding an average of 7 per cent.

The **kidneys** behave in a manner which may be considered typical for the viscera. They show a marked increase (average 90 per cent) in weight during the first period. In the next two periods the increase continues, but at a diminished rate (21-28 per cent). During the succeeding periods there is at first a slight increase (4 per cent), later a slight decrease (6-7 per cent). During adult inanition the kidneys lose considerably in weight, but somewhat less than the body as a whole.

The empty **alimentary canal** (including the stomach and intestines, with mesentery) exhibits the same tendency as the visceral group in general, with an increase in the earlier periods and a decrease later. The average increase in the second group appears rather small (17 per cent) and in the third group rather large (100 per cent), which may possibly be due to abnormal variations in the test rats or controls. During adult inanition, the loss in weight (57 per cent) is very pronounced, exceeding that in the body as a whole. The contents of the canal are also greatly decreased during adult inanition; but after the age of weaning (3 weeks) in the younger test rats the contents are usually much increased, probably due to the large amount of water taken.

The **spleen** is very irregular in its apparent tendencies, the first four groups showing alternating periods of increase and decrease. In the fifth and sixth periods the decrease is very slight. There is a marked decrease during adult inanition, especially in the acute inanition group. The irregularity of growth in the spleen during inanition recalls the fact that it is normally one of the most variable organs of the body (Jackson '13).

The **hypophysis** exhibits a typical series of reactions during underfeeding, with a definite increase during the earlier periods and a slight decrease later. There is apparently a sexual difference in the weight of the hypophysis, which (together with the histological changes) is discussed in a paper by Jackson ('17).

The **heart** likewise shows a typical series of growth reactions, with a marked tendency to increase in the earlier periods, and a slight decrease later. The small apparent decrease in the second group is possibly abnormal. During adult inanition the loss in heart weight is nearly proportional to that of the entire body.

The **ovaries** exhibit a great irregularity of growth during underfeeding. There is apparently a slight average increase (5 per cent) in the first group, a very marked increase (83 per cent) in the second group, with alternating periods of increase and decrease in the succeeding groups. These irregularities may be in part due to variations in the sexual cycle, which probably account for the excessive variability found in the normal weight of the ovaries (Jackson '13). In this connection, it may be noted that severe inanition completely prevents the normal sexual functions. The young rats (males and females) do not mature sexually during the underfeeding periods, and the females are rarely able to breed when fully refed later, even when mated with normal males.

The **suprarenal glands** exhibit in general a definite tendency to increase, which appears slight (average 5 per cent) in the first group increasing to its greatest intensity (114 per cent) in the third group. In the fourth and fifth groups the increase is smaller, and in the sixth group there appears an actual decrease (26 per cent). The suprarenals are remarkably resistant toward adult inanition, losing little or none in weight. There is also a sexual difference in the weight of the suprarenal which (together with histological changes) is discussed by Jackson ('19).

The **thyroid gland** increased slightly in the first group, and shows a slight tendency to increase (4-5 per cent) in the second and third groups, with a progressive tendency to decrease in the later periods. In adults there appears to be no loss in the thyroid weight in acute inanition and only a moderate loss (22 per cent) in chronic inanition. The histological changes in the thyroid and parathyroid glands have been described in detail by Jackson ('16).

The **lungs** show a weaker growth tendency than any of the organs hitherto considered. There is an insignificant increase (average 3 per cent) in the first group, with an apparent decrease, varying from 13 to 26 per cent, in all the succeeding periods, excepting the 3 weeks to 1 year group. It is possible that the apparent increase of 26 per cent in this group is abnormal, as lung disease is very common in the older rats. During adult inanition, the loss in lung weight is roughly proportional to that of the whole body.

The **liver** exhibits a course somewhat parallel with that of the alimentary canal, but upon a lower plane of growth impulse. In the first group, there is a definite loss of 23 per cent (average). In the second group, there is a gain of 17 per cent, which increases to a maximum of 64 per cent in the third group. The growth tendency later wanes, showing in the successive groups a gain of 10 per cent and losses of 7 and 39 per cent. In adult inanition the liver loses heavily in weight, especially in the acute inanition group (58 per cent).

The **thymus** at all stages shows a great loss in weight, in accordance with its well known tendency to atrophy during inanition (''hunger involution''). In the experiments beginning at three weeks or later, the thymus loses 90 per cent in weight. This extreme loss during inanition is paralleled only by that of the adipose tissue.

Discussion.

The growth tendencies of the various organs and systems during inanition at various periods may be compared with the normal growth rates, as worked out by Jackson and Lowrey ('12) and Jackson ('13). In the first place it may be noted that the normal growth rate in the body as a whole is relatively much greater at first, decreasing as age advances. In the rat, for example, the body weight is normally doubled during the first week after birth. To double it again requires a period nearly twice as great, and for the third doubling a period fully three times as great. Thereafter the relative growth rate gradually decreases to zero in the adult.

The normal growth rate also varies in different parts of the body. The head of the rat is relatively larger in the earlier periods, and the tail smaller. The musculature is at first relatively small, forming only 23 to 24 per cent of the body weight during the first postnatal week, the proportion gradually increasing to about 45 per cent in the adult. The skeleton, on the other hand, is relatively largest in the first week, decreasing from 17 or 18 per cent then to about 11 per cent of the body weight in the adult. Similarly the integument, which increases from 20 to 26 per cent of the body weight between birth and one week of age, thereafter decreases relatively to about 18 per cent in the adult. The visceral group as a whole increases slightly from 18 per cent of the body in the newborn to about 21 per cent of the body weight at three weeks, thereafter decreasing steadily to about 13 per cent in the adult.

Of the individual viscera, the thyroid gland is apparently relatively largest at birth, the brain, spinal cord, eyeballs, lungs and spleen at the second week, the thymus, heart, suprarenals and kidneys at three weeks, the alimentary canal and liver at about six weeks, and the gonads at about ten weeks of age. These varying degrees of relative growth under normal conditions correspond only in part with the changing growth reactions of the organs during underfeeding. In general, the various systems and organs apparently tend to grow most strongly during underfeeding at approximately those ages when they normally reach their maximum relative size. This tendency is more or less evident in the systems,—integument, musculature and visceral group as a whole. In the individual organs, the same rule would apply especially to the suprarenals, thyroid (?), liver and alimentary canal; and to a certain extent to the eyeballs, brain, spinal cord, heart and kidneys. The gonads, lungs, thymus and spleen apparently form exceptions, however. Some of the discrepancies may be due to differences in the length and severity of the various underfeeding periods, rather than the different ages at which they occur.

It is likewise evident that the tendencies to growth of the various parts during underfeeding of the young at various periods differ more or less from the tendencies of the corresponding parts to lose weight during adult inanition. It is true that some organs (skeleton, eyeballs, brain and spinal cord) which have a very persistent growth tendency throughout the younger periods of underfeeding have also a strong resistance to inanition in the adult. But there are many exceptions, especially in the earlier periods. In general, the resemblance to adult inanition increases with the age of the younger rats. It is, however, not surprising that the results of inanition should differ according to age, since the younger tissues have strong tendencies to both growth and maintenance, while in the adult only the tendency to maintenance remains.

It should moreover be emphasized that these experiments apply merely to simple inanition due to feeding restricted amounts of a wholesome, balanced diet. It is altogether probable that different forms of inanition, due to incomplete or unbalanced rations, would produce more or less different results in the various systems and organs. This, however, is an unknown field of research for future exploration.

II. Effect of Underfeeding upon Subsequent Growth during Refeeding.

Another question which arises concerns the permanence of the effects of inanition upon the growth of the young. To what extent is recovery possible upon later full refeeding? Aron ('11) found that a puppy fully refed after a year of underfeeding became very fat and increased considerably in weight, but not in stature. The growth of the skeleton appeared permanently stunted. In some later experiments on underfed rats, Aron ('14) found that they grew rapidly upon refeeding, even after growth had been repressed up to the age of 280 days, when normally the adult size would have been nearly attained. If the underfeeding period was not too extensive, the rats when fully refed finally reached their full normal size and weight. But if the underfeeding was prolonged beyond 50 to 150 days, the rats upon refeeding failed to reach their normal size and weight, thus remaining permanently stunted or dwarfed. Aron therefore concluded that full recovery upon refeeding is possible only when the retardation of growth has not extended beyond the time of the normal active growth period. He found that growth is likewise retarded by qualitative deficiencies of diet, with subsequent recovery upon normal refeeding. A proteid-poor diet appeared to affect more deleteriously the subsequent growth upon refeeding than did a corresponding degree of underfeeding with restricted amounts of a normal diet.

Brüning ('14) found that young nursing rats underfed by removal from the mother for successive periods are greatly retarded in growth. The body weight was not fully recovered upon subsequent full feeding, but the experiment was not continued long enough to determine whether the effect was permanent.

Osborne and Mendel ('14, '15), on the other hand, in an extensive series of experiments upon the growth of rats with various inadequate diets, find a remarkable capacity for full recovery upon proper refeeding, even after growth has been suppressed for periods of time (up to 550 days) far beyond the normal growth period. They claim that the capacity to resume growth does not depend upon the size or age at which the inhibition of growth is effected and state that: "It is now reasonable to ask whether the capacity to grow can ever be lost unless it is exercised." Several cases are presented to support this conclusion, chiefly those in which growth was retarded by qualitatively deficient (inadequate protein) diet. In one case, however, a female rat in which growth was repressed by "limited quantity of food," the body weight of 53 grams at 39 days reached only 59 grams at 513 days. Rapid growth ensued upon full refeeding, the body weight reaching 220 grams (which is above the usual normal maximum) in about 130 days. Unfortunately no direct controls from the same litters were kept. It is admitted that "resumption of growth has not been as perfect in every instance as in the typical records here presented," but positive results are considered more valuable, since failure may be due to various causes.

These striking results of Osborne and Mendel are very surprising and contrary to the prevailing belief that severe and prolonged underfeeding affects unfavorably the subsequent capacity for growth. On account of the fundamental importance, both theoretical and practical, of the principles involved, a very extensive and careful investigation of the subject was undertaken by Stewart. Forty-six litters of albino rats were used, including over 300 individuals. So far as possible, normal controls for each sex were kept for every litter. All were kept under the same conditions, excepting the amount of food (graham bread soaked in whole milk) allowed the test rats. In experiments before the weaning period, the test rats were underfed by removal from the mother at successive periods. Water (city supply, from the Mississippi river) was allowed *ad libitum* in all cases. The experiments have extended over a period of nearly three years. In order to determine the internal changes, complete autopsies with weights of the organs and systems were made upon normal controls as well as upon test rats after various periods of underfeeding and at the end of subsequent refeeding experiments.

The experiments may be divided into three groups as follows:

Group 1. Rats underfed from birth to three weeks (8 litters), four weeks (1 litter), six weeks (5 litters), ten weeks (6 litters), and eleven weeks (1 litter); all being subsequently fully refed for periods usually up to one year or more. The underfeeding was severe, the body weight of about 5 grams at birth being restricted so as to reach only about 10 grams at three weeks (the normal being 20 grams or more; 13 grams at six weeks (normal about 50 grams) and 15 grams at 10 weeks (normal 100 grams or more).

Group 2. Rats underfed at maintenance (constant body weight) beginning at the age of three weeks and extending to the age of four, six and ten weeks (3 litters), and twelve weeks (3 litters), with subsequent refeeding for various periods. In addition, two litters were subjected to alternating short periods of fasting and ample feeding. In general, the effects of the underfeeding were less pronounced in this group than in the others, the experiments beginning at a later age than those in the first group, and being less prolonged than those in the third group.

Group 3. Rats underfed from the age of three weeks to four months (1 litter) or to about one year of age (13 litters) and then fully refed until the maximum growth capacity was determined.

The experiments in group 2 were completed first, and the results published (Stewart '16). In general, there was obtained upon refeeding an increase in body weight even more rapid than the normal (for the same initial body weight at the earlier age), so the underfed rats upon refeeding sooner or later overtook the controls. This is shown clearly in the growth curves, the only possible question being in the males refed after seven weeks of maintenance, which seemed to lag slightly behind. These results therefore tend to confirm those of Osborne and Mendel, showing that underfeeding the young for considerable periods may not affect their capacity for later growth upon ample refeeding.

Stewart also found that not only the body weight, but also the weights of the various components organs and parts, which have been disturbed by the process of underfeeding, in this group of experiments rapidly regain their normal relative weights, usually within a period of four weeks.

Quite different results, however, were obtained in the first and third groups, in which the experiments have just been completed, the results being hitherto unpublished. In the first group, the underfeeding began at birth. In those refed after reaching the age of three weeks, the body weight reached that of the controls in only a few exceptional cases. Even in these cases, the result was apparently due to some unhealthy condition which prevented the control rat from reaching the usual normal growth in body weight. *In no case in which the underfeeding from birth was prolonged beyond three weeks did the test rats upon ample refeeding reach the maximum body weight of the normal controls in the same litter!*

The following cases may be cited as typical for the first group:

Litter No. St 122. Five males and four females. One of each sex full fed for controls. Remainder underfed from birth to three weeks, with increase in body weight from about 6 grams to 10.7 grams; then fully refed. Of the seven test rats, five died at various periods. Of the two survivors, one male (No. 8) reached a maximum body weight of 230 grams, and one female (No. 1) reached 208 grams. The normal control male (No. 2) reached 345 grams, the female (No. 6) 248 grams.

Litter No. St 71. Four males and four females. The males all died during the experiment. Of the females, the full fed control (No. 7) reached a maximum body weight of 175 grams. The three test females were underfed from birth (body weight 6 grams) to six weeks of age (body weight 13 grams), two of the rats dying during the experiment. The survivor (No. 2) reached a maximum body weight of 158 grams upon refeeding.

Litter No. St 76. Six males and five females. All the females and three males died during the experiment. Two full fed control males (No. 6 and No. 8) reached maximum body weights of 280 and 310 grams, respectively. One test male (No. 7) was underfed from birth (at 6.8 grams) to six weeks of age (15.6 grams) and then fully refed, reaching a maximum body weight of 171 grams.

Litter No. St 65. Seven males and five females. Two controls were fully fed, one male (No. 6) reaching a maximum of 217 grams and one female (No. 16) 196 grams body weight. The ten test rats were underfed from birth (weight about 5 grams) to ten weeks of age (body weight about 15 grams) and then fully refed. Of three survivors, one male (No. 4) reached a maximum of 158 grams, one male (No. 9) 138 grams and one female (No. 2) 117 grams body weight.

In the third group experiments, in which the underfeeding began (as in the second group) at three weeks of age, but was prolonged over longer periods, up to the age of four months to about one year, the results were even more striking than in the first group. *In not a single case did the maximum body weight upon refeeding reach that of the normal controls in the same litter!*

The following may be cited as typical cases from the third group:

Litter No. S 33. Four males, six females. Of the two fully fed controls, one male (No. 117) reached a maximum body weight of 320 grams and one female (No. 116) reached 233 grams. The eight test rats were underfed from the age of three weeks (body weight about 20 grams) to age of four months (body weight about 36 grams). Four of the test rats died before the refeeding began, and one shortly afterward. Of the three survivors, two males (Nos. 118 and 119) reached maximum body weights of 233 and 236 grams, and one female (No. 120) reached 162 grams.

Litter No. St 29. Four males and seven females. Of the two controls, one male (No. 167) reached a maximum of 290 grams, one female (No. 169) 184 grams in body weight. The nine test rats were underfed from the age of three weeks (body weight about 21 grams) to the age of eleven months (body weight 36 to 59 grams). Five of the test rats were now dead, and two more died shortly after refeeding began. Of the two survivors, one male (No. 172) reached a maximum body weight of 157 grams, and one female (No. 166) reached 129 grams.

More complete details concerning the results, together with a consideration of the individual organs and parts, will be published separately.

It, therefore, appears that the suppression of growth in young rats affects their subsequent capacity to grow according to the age of the rats and the length and severity of the underfeeding. After the age of weaning (three weeks) the rats may be held at maintenance (constant body weight) by underfeeding for several weeks, with prompt recovery of the normal maximum body weight upon refeeding. A similar period of underfeeding beginning at birth, however, results in a permanent stunting of the body, which is incapable of recovery. The same is true of rats underfed beginning at three weeks of age, in cases where the underfeeding is prolonged over several months. In general, it appears that the injurious effect is inversely proportional to the age of the animal and directly proportional to the length of the underfeeding experiment.

These results, therefore, tend to confirm the conclusions of Aron and Brüning, opposing those of Osborne and Mendel. The more favorable results of the latter may be due, in the first place, to the difference in the method of underfeeding. It is quite possible that various inadequate protein diets fed in abundant amounts might have less injurious effects upon subsequent growth capacity than does the feeding of restricted amounts of a balanced ration, though Aron reached the opposite conclusion.

The apparently different results may also be explained in another way. Individual rats (and perhaps individual strains) vary greatly in their resistance toward inanition. In Osborne and Mendel's experiments, it seems that a few individuals were able to maintain their capacity for growth under conditions which would in most cases produce permanent stunting. To what extent this is true in their experiments is uncertain, since they do not state the total number of test animals from which the successful cases were selected. In our experience, as noted above, a large percentage of the animals die during the severe underfeeding experiments, either directly from inanition or indirectly through lowered resistance to disease. Practically all of the survivors are permanently stunted in their ultimate capacity for growth, although some upon autopsy reveal no evident disease. There seems no escape from the conclusion that while a considerable degree of inanition may be followed by prompt and complete growth recovery upon ample refeeding, more severe and prolonged underfeeding, especially in very young individuals, reduces materially their capacity for subsequent growth.

LITERATURE CITED.

Aron, Hans: 1910, Wachstum und Ernährung. Bio-Chemische Zeischrift, Bd. 30, S. 207-226.

—— 1911, Nutrition and Growth. Philippine Journal of Science, Vol. 6, Feb.

—— 1914, Untersuchungen über die Beeinflussung des Wachstums durch die Ernährung. Berl. klin. Wochenschrift, J. 51, No. 21, S. 972-977.

Birk, W.: 1911, Unternährung und Längenwachstum beim neugeborenen Kinde. Berl. klin. Wochenschrift, J. 48, Bd. 2, S. 1227-1231.

Brüning, Herman: 1914, Experimentelle Studien über die Entwicklung neugeborener Tiere bei längerdauernder Trennung von der säugenden Mutter und nachheriger verschiedenartiger künstlicher Ernährung. Jahrb. f. Kinderheilk., Bd. 80, S. 65-85.

Donaldson, H. H.: 1915, The Rat. Reference Tables and Data. Memoirs of the Wistar Institute of Anatomy and Biology, No. 6, Philadelphia.

Freund, W.: 1909, Zur Pathologie des Längenwachstums bei Säuglingen. Jahrb. f. Kinderheilk. Bd. 70, S. 752. (Cited by Birk '11 and Brüning '14.)

Hess, Alfred F.: 1916, Infantile scurvy. III. Its Influence on Growth (Length and weight). Amer. Jour. Diseases of Children, Vol. 12, p. 152-165.

Jackson, C. M.: 1913, Postnatal Growth and Variability of the Body and of the Various Organs in the Albino Rat. Amer. Jour. Anatomy, Vol. 15, p. 1-68.

—— 1915, Effects of acute and Chronic Inanition upon the Relative Weights of the Various Organs and Systems of Adult Albino Rats. Amer. Jour. Anatomy, Vol. 18, p. 75-118.

Jackson, C. M.: 1915a, Changes in the Relative Weights of the Various Parts, Systems and Organs of Young Albino Rats Held at Constant Body Weights by Underfeeding for Various Periods. Jour. Exper. Zoology, Vol. 19, p. 99-156.

—— 1916, Effects of Inanition upon the Structure of the Thyroid and Parathyroid Glands of the Albino Rat. Amer. Jour. Anatomy, Vol. 19, p. 305-352.

—— 1917, Effects of Inanition and Refeeding upon the Growth and Structure of the Hypophysis in the Albino Rat. Amer. Jour. Anatomy, Vol. 21, p. 321-358.

—— 1919, The Postnatal Development of the Suprarenal Gland and the Effects of Inanition upon its

Growth and Structure in the Albino Rat. (In press, Amer. Jour. Anatomy, to appear in next Jan. or March.)

Jackson, C. M. and Lowrey, L. G.: 1912, On the relative Growth of the Component Parts (Head, trunk and Extremities) and Systems (Skin, Skeleton, Musculature and Viscera) of the Albino Rat. Anat. Record, Vol. 6, p. 449-474.

Osborne, Thomas B. and Mendel, Lafayette B.: (with the Co-operation of Edna L. Ferry and Alfred J. Wakeman): 1914, The Suppression of Growth and the Capacity to Grow. Jour. Biol. Chem., Vol. 18, No. 1. Also, 1915, Jour. Biol. Chem., Vol. 23, No. 2.

Stewart, Chester A.: 1916, Growth of the Body and of the Various Organs of Young Albino Rats after Inanition for Various Periods. Biol. Bulletin, Vol. 31, p. 16-51.

—— 1918, Changes in the Relative Weights of the Various Parts, Systems and Organs of Young Albino Rats Underfed for Various Periods. Jour. Exper. Zoology, Vol. 25, p. 301-353.

—— 1918a, Weights of Various Parts of the brain in normal and underfed Albino Rats at Different Ages. (In press. Jour. Exper. Neurology, October.)

—— 1918b, Changes in the Weights of the Various Parts, Systems and Organs in Albino Rats Kept at Birth Weight for Various Periods. (Ready for Publication.)

Variot: 1907 and 1908, La Clinique Enf. 15. XII and 1. V. (Cited by Brüning '14.)

—— 1907, La dissociation de la croissance dans l'atrophie et l'hypotrophie infantiles; atrophie ponderale, atrophie staturale. Bull. et Mém. Soc. d. Hôp. de Paris, T. 24, p. 1347-1376. Also in Bull. Soc. de la Pédiatr. de Paris, T. 9, p. 363-369.

Waters, H. J.: 1908, Capacity of Animals to Grow under Adverse Conditions. Proc. Soc. for the Promotion of Agricultural Science.

—— 1908a, The Influence of Nutrition upon the Animal Form. Presented at the Thirtieth Meeting of the Society for the Promotion of Agricultural Science.

No. 6

Recovery of Normal Weight in the Various Organs of Albino Rats on Refeeding After Underfeeding from Birth for Various Periods

---

C. M. JACKSON, M.S., M.D.
AND
C. A. STEWART, Ph.D.
MINNEAPOLIS

3

CONTENTS

PAGE
Introduction 1
Material and methods 6
Body weight 10
Body length and tail length 12
Head, limbs and trunk 13
Integument 13
Skeleton 14
Musculature 15
Visceral group and "remainder" 15
Brain 16
Spinal cord 17
Eyeballs 18
Hypophysis and pineal body 18
Thyroid gland 19
Thymus 20
Lungs 20
Liver 21
Spleen 21
Stomach and intestines 22
Suprarenal glands 24
Kidneys 24
Testes and epididymides 24
Ovaries 25
Discussion 25
Summary 27

5

RECOVERY OF NORMAL WEIGHT IN THE VARIOUS ORGANS OF ALBINO RATS ON REFEEDING AFTER UNDERFEEDING FROM BIRTH FOR VARIOUS PERIODS *

C. M. JACKSON, M.S., M.D., AND C. A. STEWART, PH.D.
MINNEAPOLIS

Previous research by ourselves and others (summarized by Jackson and Stewart,[1] 1918) has shown that in young animals underfed for various periods remarkable changes occur in the weights of the various organs and parts of the body. There is also evidence indicating that similar changes occur in malnourished human infants. While some organs during inanition tend to maintain approximately their normal relative weight, and others continue to grow, still others undergo losses. The organs affected and the extent of the changes involved vary according to the age of the individual and the length and character of the inanition.

The question naturally follows as to the process of recovery on abundant refeeding after various periods of inanition. Stewart[2] has already shown that rapid recuperation occurs in rats refed after being held at maintenance (constant body weight) from the age of weaning (3 weeks) to about the age of puberty (10 or 12 weeks). The various organs and parts usually recover their normal relative weights within four weeks of refeeding. The object of the present investigation is to determine the extent of recovery on similar refeeding of rats which have been underfed from birth, during a period in which the changes due to underfeeding are much greater than at later periods (Stewart,[3] 1918, 1919). The results may perhaps indicate the probability of recovery in the various organs of infants after periods of inanition, a clinical problem of obvious importance.

---

* From the Institute of Anatomy, University of Minnesota.

[1] Jackson, C. M., and Stewart, C. A.: The Effects of Underfeeding and Refeeding on the Growth of the Various Systems and Organs of the Body, Minnesota Med. **1**:403, 1918.

[2] Stewart, Chester A.: Growth of the Body and of the Various Organs of Young Albino Rats after Inanition for Various Periods, Biol. Bull. **31**:16, 1916.

[3] Stewart, Chester A.: Changes in the Relative Weights of the Various Parts, Systems and Organs of Young Albino Rats Underfed for Various Periods, J. Exper. Zool. **25**:301, 1918. Ibid. Changes in the Weights of the Various Parts, Systems and Organs in Albino Rats Kept at Birthweight for Various Periods, Am. J. Physiol. **48**:67, 1919.

MATERIAL AND METHODS

The albino rat (*Mus norvegicus albinus*) was used as heretofore, including complete data for 31 test rats (15 males, 16 females) and 22 controls (9 males, 13 females), the individuals being listed in Table 1. For intestinal lengths, 45 additional test rats (26 males, 19 females) and 24 controls (12 males, 12 females) were used, corresponding chiefly to the same individuals used in previous papers by Stewart[3] (1918 and 1919). The letters "St" (Stewart) refer to the series, the number preceding the decimal point refers to the litter and the number following designates the individual rat. Thus, it is apparent that, for the most part, the test rats and controls were from the same litters. This is a matter of importance, especially in view of the comparatively small number of animals used, since it has been shown by Jackson[4] and King[5] that variability in weight within the litter is very much less than that of the rat population in general.

The rats were well cared for in the animal rooms of the Institute of Anatomy, and were kept as nearly as possible under uniform environment, varying only the nutritive conditions as desired. The necropsies were performed and the data carefully recorded by Stewart, the technic being the same as that described in our previous articles.

The test rats were all underfed by removal from the mother for various successive periods from birth up to the period of weaning (about three weeks), and later by feeding restricted amounts of whole wheat (Graham) bread, soaked in whole milk. Water (city supply, from Mississippi river) was also furnished. Some of the rats were underfed from birth to 3 weeks of age. The gross body weight during this time increased from about 5 gm. to only about 10 gm., whereas normally it would have increased to 20 or 25 gm. Some were similarly underfed from birth to 6 weeks (exact average being 45 days), reaching an average weight of 13 to 14 gm. (normal at this time being about 50 gm). In some, the underfeeding was prolonged from birth to the age of 10 weeks (66 days in group E, 67 days in group F) the body weight reaching about 16 gm. (normal about 100 to 120 gm.). These three groups were therefore in the same condition as those in which Stewart[3] (1918) has determined the changes in the weights of the organs and parts at the end of the underfeeding period.

The first group (underfed from birth to 3 weeks), shown in Tables 1 and 2, were then placed on a full diet of bread and milk, and refed until they had reached body weights of approximately 25 gm., 50 gm. or 75 gm., respectively. The second group (underfed to 6 weeks) were similarly refed until they had reached a body weight of about 75 gm. The third group (underfed to 10 weeks) were similarly refed, some to about 50 gm. and the others to about 75 gm., as shown in Tables 1 and 2.

The normal controls were likewise killed in three groups, with approximate gross body weights of 25, 50 and 75 gm., respectively, corresponding to the *final* body weights of the various test groups.

It is thus possible to compare directly the weights of organs in the controls and in the test groups, and to ascertain in each case to what extent they approach the normal for corresponding body weight. The difference is expressed in Table 2 as a *percentage* (plus or minus) in each case. The correction for differences in body weight between control and test groups is so slight that it was ignored. There is also

---

[4] Jackson, C. M.: Postnatal Growth and Variability of the Body and of the Various Organs in the Albino Rat, Am. J. Anat. **15**:1, 1913.

[5] King, Helen D.: The Growth and Variability in Body Weight of the Albino Rat, Anat. Rec. **9**:751, 1915.

TABLE 1.—ALBINO RATS USED. INDIVIDUAL DATA FOR SEX, AGE, GROSS BODY WEIGHT AND BODY LENGTH

	Rat Number	Sex	Age, Days	Gross Body Weight, Gm.	Nose-Anus Length, Mm.
Normal controls	St 95.4	M	20	24.4	88
	St 104.6	M	23	26.2	90
	St 131.1	M	22	26.4	89
	St 157.6	M	22	29.6	98
	St 98.1	F	21	22.4	87
	St 104.5	F	23	26.5	90
	St 106.3	F	20	25.5	91
	St 132.5	F	20	28.0	93
	Average		21.4	26.1	90.8
Underfed from birth to 3 weeks and refed	St 106.2	M	35	25.0	98
	St 131,2	M	29	27.2	96
	St 131.4	M	29	25.0	95
	St 150.4	M	32	25.0	93
	St 106.1	F	33	25.6	95
	St 128.1	F	33	26.5	97
	St 132.3	F	34	25.3	96
	St 132.6	F	34	27.5	98
	St 150.1	F	32	25.6	95
	Average		32.3	25.9	95.9
Normal controls	St 154.2	M	42	55.4	126
	St 107.6	M	35	50.5	121
	St 109.4	M	37	52.8	120
	St 167.2	M	30	51.5	119
	St 153.1	F	48	49.5	119
	St 153.6	F	37	44.5	116
	St 171.3	F	27	49.0	118
	St 154,5	F	45	56.5	121
	St 152.1	F	43	50.0	124
	Average		38.2	51.1	120.4
Underfed from birth to 3 weeks and refed	St 144.5	M	51	49.6	130
	St 144.6	M	51	49.5	123
	St 152.4	M	51	52.0	120
	St 144.1	F	54	53.3	126
	St 144.2	F	59	53.5	123
	St 144.4	F	48	50.0	124
	St 152.3	F	52	51.5	122
	St 152.7	F	51	50.0	117
	St 152.9	F	49	50.0	120
	Average		51.6	51.0	122.8
Underfed from birth to 66 days and refed	St 158.8	F	77	46.9	119
	St 154.7	F	82	51.7	121
	St 159.3	F	82	52.0	128
	St 153.10	F	81	51,1	123
	Average		80.5	51.7	122.8
Normal controls	St 91.9	M	48	76.0	141
	St 143.3	M	46	77.0	140
	St 155.2	F	53	73.0	137
	St 168.1	F	50	75.5	140
	St 170.1	F	46	80.3	135
	Average		48.6	76.4	138.6
Underfed from birth to 3 weeks and refed	St 137.4	M	61	76.6	141
	St 137.6	M	61	75.5	136
	St 143.1	M	54	75.5	140
	St 143.9	M	56	78.5	145
	St 147.1	M	67	77.0	140
	St 147.4	F	94	74.8	141
	Average		65.5	76.3	140.5
Underfed from birth to 45 days and refed	St 91.4	M	89	80.5	135
	St 91.8	M	96	74.0	133
	Average		92.5	77.3	134.0
Underfed from birth to 67 days and refed	St 154.3	F	101	75.5	139.0

TABLE 2.—DATA FOR VARIOUS PARTS AND ORGANS; AVERAGES FOR GROUPS OF RATS, ACCORDING TO TESTS, WITH CORRESPONDING NORMAL CONTROLS AND PERCENTAGE DIFFERENCE

Description	A. Underfed from Birth to 3 Weeks of Age and Refed to about 25 Gm. (with Controls)			B. Underfed from Birth to 3 Weeks of Age and Refed to about 50 Gm. (with Controls)			C. Underfed from Birth to 3 Weeks of Age and Refed to about 75 Gm. (with Controls)			D. Underfed from Birth to 6 Weeks (45 days) of Age and Refed to about 75 Gm. (with Controls)			E. Underfed from Birth to 10 Weeks (66 days) of Age and Refed to about 50 Gm. (with Controls)			F. Underfed from Birth to 10 Weeks (67 days) of Age and Refed to about 75 Gm. (with Controls)		
	Controls	Test Rats	Difference	Controls	Test Rats	Difference	Controls	Test Rats	Difference	Controls	Test Rats	Difference	Controls	Test Rats	Difference	Controls	Test Rats	Difference
Number and sex	4M, 4F	4M, 5F	......	4M, 5F	3M, 6F	......	2M, 3F	5M, 1F	......	2M	2M	......	5F	4F	......	3F	1F	
Age, days	21.4	32.3	......	38.2	51.6	......	48.6	65.5	......	47.0	92.5	......	40.0	80.5	......	49.7	101.0	
Body weight, Gm.:																		
Gross	26.1	25.9	—0.8	51.1	51.0	—0.2	76.4	76.3	—0.1	76.5	77.3	+1.0	45.7	47.9	+4.8	69.2	71.2	+2.9
Net	24.2	24.1	—0.4	47.2	47.5	+0.6	70.1	71.6	+2.1	72.0	71.3	—0.1	49.9	51.7	+3.6	76.3	75.5	—0.1
Nose-anus lenth, Mm.	90.8	95.9	+6.0	120.4	122.8	+2.0	138.6	140.5	+1.4	140.5	134.0	—4.6	119.6	122.8	+2.7	137.3	139.0	+1.2
Tail lenth, Mm.	60.0	63.0	+5.0	95.3	96.7	+1.5	116.0	109.0	—6.0	111.0	113.0	+1.8	96.0	90.0	—6.3	118.0	111.0	—5.9
Head, Gm.	4.84	4.56	—5.8	6.89	6.50	—5.7	8.68	8.70	0.0	8.75	8.75	0.0	6.80	6.43	—5.4	8.63	8.40	—2.7
Fore limbs, Gm.	1.79	1.87	+4.5	3.54	3.29	—7.1	4.96	5.17	+3.8	4.97	4.71	—5.6	3.45	3.45	0.0	4.95	5.61	+13.3
Hind limbs, Gm.	3.39	3.56	+5.0	7.54	7.57	+0.4	11.50	11.73	+2.0	11.61	11.18	—3.7	7.40	7.08	—4.3	11.43	10.62	—7.1
Trunk, Gm.	14.17	14.14	—0.2	29.00	30.16	+4.0	45.20	46.51	+2.9	46.67	46.63	0.0	27.67	30.92	+11.8	44.23	46.55	+5.2
Integument, Gm.	4.89	3.91	—20.1	8.81	8.49	—3.6	13.36	13.75	+2.9	14.06	14.65	+4.2	8.37	9.21	+10.0	12.90	17.06	+32.3
Skeleton:																		
Ligament, Gm.	3.77	4.04	+7.2	6.34	5.91	—6.8	7.98	8.24	+3.3	8.46	7.89	—6.7	6.15	5.76	—6.3	7.66	8.07	+5.4
Moist cartilage, Gm.	3.04	2.87	—5.6	5.51	5.00	—9.3	7.20	7.06	—1.9	7.19	5.80	—19.3	5.39	3.97	—26.3	7.23	7.75	+7.2
Dry cartilage, Gm.	0.768	0.790	+2.9	1.80	1.59	—11.7	2.56	2.41	—5.9	2.51	1.81	—27.9	1.89	......	......	2.68		
Musculature, Gm.	6.98	7.31	+4.7	16.12	15.94	—1.1	26.84	27.71	+3.2	28.08	26.47	—5.7	15.79	13.36	—15.4	26.01	24.05	—7.5
Visceral group, Gm.	5.39	5.51	+2.2	8.88	9.32	+5.0	12.71	12.25	—3.6	13.68	12.04	—12.0	8.84	9.90	+12.0	12.07	13.12	+8.7
Remainder, Gm.	3.18	3.24	+1.9	7.05	7.93	+12.5	9.42	9.65	+2.4	7.67	10.22	+33.2	6.59	10.39	+57.6	10.59	8.89	—16.0
Brain, Gm.	1.331	1.162	—12.7	1.496	1.252	—16.3	1.488	1.314	—11.7	1.500	1.437	—4.2	1.446	1.120	—22.5	1.479	1.244	—15.9
Spinal cord, Gm.	0.184	0.194	+5.4	0.279	0.261	—6.5	0.317	0.311	—1.3	0.323	0.321	—0.6	0.268	0.248	—7.5	0.313	0.278	—11.2
Eyeballs, Gm.	0.117	0.128	+9.5	0.161	0.158	—1.9	0.177	0.179	+1.1	0.173	0.206	+19.1	0.161	0.170	+5.6	0.179	0.194	+8.4
Hyphophysis, Gm.	0.0024	0.0024	0.0	0.0031	0.0028	—10.0	0.0044	0.0039	—11.4	0.0042	0.0048	+14.3	0.0030	0.0034	+13.3	0.0046	0.0041	—10.9
Pineal body, Gm.	0.00100	0.00094	—6.0	0.00114	0.00102	—10.5	0.00108	0.00102	—5.6	0.00110	0.00115	+4.6	0.00118	0.00098	—17.0	0.00107	0.00080	—25.2
Thyroid Gland, Gm.	0.0074	0.0064	—13.5	0.0090	0.0076	—15.6	0.0111	0.0117	+7.2	0.0142	0.0149	+4.9	0.0076	0.0073	—4.0	0.0091	0.0097	+6.6
Thymus, Gm.	0.079	0.076	—3.8	0.152	0.174	+14.5	0.263	0.233	—11.4	0.275	0.201	—26.9	0.131	0.109	—16.8	0.256	0.260	+1.6
Heart, Gm.	0.181	0.177	—2.2	0.285	0.268	—6.0	0.374	0.373	0.0	0.380	0.385	+1.3	0.264	0.315	+19.3	0.370	0.421	+13.8
Lungs, Gm.	0.277	0.281	+1.4	0.406	0.355	—12.6	0.482	0.489	+1.5	0.475	0.494	+4.0	0.363	0.429	+18.2	0.485	0.635	+30.9
Liver, Gm.	1.36	1.35	—0.7	2.41	2.79	+15.8	4.16	3.75	—9.9	4.19	4.18	—0.2	2.51	2.96	+17.9	4.14	3.84	—7.3
Spleen, Gm.	0.094	0.143	+52.1	0.219	0.293	+33.8	0.452	0.450	0.0	0.337	0.255	—24.3	0.236	0.500	+112.0	0.528	0.468	—11.4
Stomach and Intestines:																		
With contents, Gm.	3.24	3.32	+2.5	6.57	6.58	+0.1	9.75	8.47	—13.1	8.60	9.10	+5.8	7.07	7.24	+2.4	10.52	9.16	—13.0
Empty, Gm.	1.31	1.58	+20.6	2.68	3.05	+13.8	3.71	3.75	+1.1	4.04	3.11	+23.0	2.89	3.40	+17.7	3.49	4.83	+38.4
Suprarenals, Gm.	0.0091	0.0093	+2.2	0.0148	0.0137	—7.4	0.0180	0.0178	—1.1	0.0172	0.0190	+10.5	0.0143	0.0144	+0.7	0.0185	0.0215	+16.2
Kidneys, Gm.	0.347	0.335	—3.5	0.561	0.511	—8.9	0.792	0.736	—7.0	0.804	0.801	—0.4	0.530	0.625	+17.9	0.784	0.899	+14.7
Testes, Gm.	0.120	0.141	+17.5	0.375	0.447	+19.2	1.047	0.579	—44.6	1.047	0.528	—49.4						
Epididymides, Gm.	0.025	0.026	+4.0	0.055	0.056	+1.8	0.108	0.079	—26.9	0.108	0.090	—16.7						
Ovaries, Gm.	0.0094	0.0115	+22.3	0.0108	0.0104	—3.7	0.0140	0.0162	+15.8	......	......	......	0.0108	0.0086	—20.4	0.0140	0.0100	—28.6

a slight error involved in computing the percentages from the *average* for the group, rather than for each individual separately. Finally, of course, due allowance must be made for experimental error and normal variability, in view of the comparatively small number of observations in each group. These considerations make it necessary to be cautious in drawing final conclusions, although the results in general seem to justify some provisional conclusions, as will appear later.

If the data for the normal control rats in the present series are compared with the normal tables of Donaldson,[6] derived from the formulas of Hatai,[7] a general agreement is found, although certain differences appear. As we have repeatedly noted, in the normal rats from the Minnesota and Missouri colonies, the weight of the liver is constantly lower, and that of the spleen higher, than the Wistar norm for rats of the same body length or weight (Jackson,[4] Stewart,[2, 3] 1916 and 1918). In the present series, the body length in the normal rats averages slightly below the Wistar norm for animals of the same body weight, that is, our animals are relatively a little heavier for the same body length. The tails are likewise somewhat shorter in our series. The thymus is heavier than the Wistar norm for the corresponding age, usually also for the corresponding body weight. The empty stomach and intestines are lighter, and the ovaries somewhat heavier, than the Wistar norm. In the other organs, there is but slight or inconstant difference. It is uncertain whether the above differences mentioned are accidental (due to chance variation in the small series), environmental (due to differences in diet, temperature, etc.), or germinal (due to different varieties or strains of albino rats). At any rate, they emphasize the necessity of having direct controls in experimental work of this kind. The Wistar norm is, of course, also exceedingly valuable as a common standard, whereby variations in local strains may be recognized and compared.

In Table 1, the individual (gross) body weights and body lengths are given, which will show the variations within each group. For economy of space, only the averages are given in each group for the other data (Tables 2 and 3). The normal variability and correlation in the weight of the various organs has been studied by Jackson.[4] The individual data in the present series, as heretofore, will be deposited in the Wistar Institute of Anatomy, Philadelphia, where they will be available to those interested.

---

[6] Donaldson, H. H.: The Rat. Reference Tables and Data for the Albino Rat and the Norway Rat, Memoirs Wistar Inst. Anat. and Biol. 1915, No. 6.

[7] Hatai, S.: On the Weights of the Abdominal and Thoracic Viscera, the Sex Glands, Ductless Glands and the Eyeballs of the Albino Rat (*Mus norvegicus albinus*) According to body weight, Am. J. Anat. **15**:87, 1913.

TABLE 3.—AVERAGE LENGTH OF LARGE AND SMALL INTESTINES IN THE VARIOUS GROUPS OF ALBINO RATS *

	Age, Days	Number and Sex	Gross Body, Weight, Gm.	Body Length, Mm.	Length of Intestine		Ratio of Intestine to Body Length	Ratio of Small to Large Intestine
					Large, Mm.	Small, Mm.		
A. Normal controls	1	1 M, 1 F	4.3	46(est.)	20.0	179	4.3	9.0
	9	6 M, 6 F	10.0	63	41.8	307	5.5	7.4
	13	3 M, 3 F	15.5	74	45.7	355	5.4	7.8
	21	3 M, 5 F	25.8	90	66.3	501	6.3	7.6
	39	3 M, 4 F	50.9	121	99.9	682	6.5	7.8
	49	2 M, 3 F	76.4	139	117.0	892	7.3	7.6
B. Underfed from birth to age indicated	19	2 M, 2 F	5.0	56.0 (+22)	36.2 (+81)	239 (+25)	4.9	6.6
	22	15 M, 9 F	10.1	68.0 (+8)	48.3 (+15)	349 (+14)	5.8	7.2
	42	4 M, 3 F	13.7	79.3	64.3	506	7.2	7.9
	67	7 M, 4 F	15.3	85.3 (+15)	67.3 (+47)	553 (+56)	7.3	8.2
C. Refed after underfeeding from birth to 3 weeks of age	33	4 M, 5 F	25.9	96.0 (+6)	76.4 (+15)	560 (+12)	6.6	7.3
	52	3 M, 5 F	50.8	122.4 (+1)	97.0 (—3)	745 (+9)	6.9	7.7
	66	5 M, 1 F	76.3	140.5 (+1)	105.3 (—10)	832 (—7)	6.7	7.9
D. Refed after underfeeding from birth to 6 weeks of age	93	2 M	77.3	134 (—4)	122.5 (+5)	877 (—2)	7.5	7.2
E. Refed after underfeeding from birth to 10 weeks of age	81	4 F	51.9	123 (+2)	107.3 (+7)	802 (+18)	7.4	7.5
	101	1 F	75.5	139 (+0)	114.0 (—3)	798 (—10)	6.6	7.0

* Figures in parenthesis indicate percentage changes of corresponding part, in comparison with normal controls of nearly the same body weight.

Body Weight.—The average time required to reach the body weights of 25, 50 and 75 gm. on refeeding in the various groups may be learned from the data in Table 2. Thus, Group A, underfed from birth to three weeks, required an average of 11.3 days (final age 32.3 days) of ample refeeding to reach a body weight of about 25 gm. Similarly to reach about 50 (51) gm. (Group B) required 30.6 days, and to reach 75 (76.3) gm. (Group C) required 44.5 days of refeeding.

In Group D, underfed from birth to about 6 weeks (45 days), 47.5 days of ample refeeding were required to reach a final body weight of about 75 (77.3) gm.

In Group E, underfed from birth to about 10 weeks (66 days), only 14.5 days of ample refeeding were required to reach a body weight of 50 gm. This is astonishingly rapid growth, in comparison with the time required for Group B to reach the same body weight. In Group F, similarly underfed to ten weeks (67 days), only 34 days of refeeding were required to reach a body weight of 75 gm. This is likewise a shorter time than that required to reach the same body weight by Groups C and D, which had been underfed for shorter periods.

This more rapid recovery of body weight in the groups underfed to ten weeks of age is in part explained by the fact that they had reached a somewhat higher body weight at the end of the underfeeding period. Some time would naturally be required on refeeding to enable the rats at 10 or 13 gm. body weight to reach the initial weight of about 16 gm. which had been attained by Groups E and F at the end of the underfeeding period.

But this difference in initial body weight is insufficient to explain the shorter time of refeeding required for Groups E and F. Even when all due allowance is made, the surprising fact remains that the recovery in body weight after the longer underfeeding period is more rapid than after the shorter underfeeding periods. This, of course, might be due to an accidental variation, as individuals differ considerably in their resistance to inanition and in power of recuperation. That it is not merely an accidental variation, however, is indicated by the somewhat similar results of Stewart,[2] who found that rats amply refed after maintenance by underfeeding from 3 to 6, 8 or 10 weeks of age actually grow more rapidly than normally, so as to overtake later the normal controls of the same age. In Stewart's series it also appears that for a time the growth on refeeding was slightly more rapid in the groups (especially the females) underfed to 10 weeks (compare his Charts A and B). This surprising phenomenon of more rapid growth on refeeding does not necessarily mean that those underfed for the longer periods will ultimately reach full normal body weight, although this apparently happened in most of Stewart's earlier series (Jackson and Stewart[1]). It furthermore does not necessarily mean that the various organs and parts of the body have more quickly regained their normal proportions in the groups refed after the longer underfeeding periods. As will be shown later, this is frequently not the case.

The phenomenon of unusually rapid growth following periods of growth suppression has previously been noted by Hatai,[8] Ferry,[9] and Osborne and Mendel[10] in the rat, as well as in other animals (see citations by Stewart[2]). A similar phenomenon has likewise been noted in children by Coudereau,[11] Pagliani,[12] Schloss,[13] Boas[14] and others.

---

[8] Hatai, S.: Effect of Partial Starvation, Followed by a Return to Normal Diet, on the Growth of the Body and Central Nervous System of Albino Rats, Am. J. Physiol. **18**:309, 1907.

[9] Ferry, Edna L.: The Rate of Growth of the Albino Rat, Anat. Rec. **7**: 1913, No. 12.

[10] Osborne, T. B., and Mendel, L. B.: The Resumption of Growth after Long-Continued Failure to Grow, J. Biol. Chem. **23**: 1915. Ibid. Acceleration of Growth after Retardation, Am. J. Physiol. **40**: No. 1, 1916.

[11] Coudereau: Récherches chimiques et physiologiques sur l'alimentation des enfants, Paris, 1869. (Cited by Hatai, Footnote 8.)

[12] Pagliani: Giornale della reale Società italiana d'igiene, Milano **1**: 1879. (Cited by Hatai, Footnote 8.)

[13] Schloss, E.: Die Pathologie des Wachstums im Säuglingsalter, Berlin, 1911.

[14] Boas, F.: The Growth of Children, Science, New Series **36**:815, 1912.

Body Length and Tail Length.—The body length is measured from the tip of the nose to the anus, the trunk being slightly extended. Stewart[3] (1918) found that in rats underfed from birth (to the same extent as those of the present series) the body length had increased in the test rats, as compared with normal controls of the same body weight. The increase amounted to 6 per cent. in those underfed to the age of 3 weeks, 9 per cent. in those underfed to 6 weeks, and 13 per cent. in those underfed to 10 weeks.

As shown by the data in Table 2, the rats of the present series on ample refeeding after the various inanition periods rapidly regain their normal weight-length proportions. In those underfed from birth to 3 weeks of age (body weight about 10 gm.) and refed to a body weight of 25 gm., the body (nose-anus) length has apparently not yet reached its normal ratio, however, being still 6 per cent. greater than in the normal controls. Rats of the same group refed to body weights of 50 and 75 gm. show normal lengths, however, the difference of 2 per cent. being doubtless within the range of normal variation. The differences in the groups refed after underfeeding to 6 and 10 weeks are probably likewise of no significance.

The tail was found by Stewart[3] (1918) to become relatively elongated in rats underfed from birth to 3, 6 or 10 weeks of age. His data show an average increase of about 44 per cent., 38 per cent., and 61 per cent., respectively, as compared with normal controls of the same body weight.

As is evident from our Table 2, such rats, on ample refeeding to a body weight of 25 gm. or more, rapidly recover the normal tail ratio. The plus or minus differences do not exceed 5 or 6 per cent., and are probably within the range of normal variation. Although there is a difference in tail length according to sex (the tail in the female being slightly longer), a grouping of the data according to sex leads to the same conclusion as to recovery of the normal tail length. The sexes are, therefore, combined in the table.

A similar elongation of the body and tail was observed in rats held at maintenance (constant body weight), beginning at 3 weeks of age, by Jackson,[15] who noted that the tail normally undergoes relative elongation in the growing young rats at this period. Stewart[2] found that the abnormal body length and tail ratio in rats held at maintenance from the age of 3 to 12 weeks disappears rapidly on ample refeeding, the normal proportions usually being regained within one week.

---

[15] Jackson, C. M.: Changes in the Relative Weights of the Various Parts, Systems and Organs of Young Albino Rats Held at Constant Body Weight by Underfeeding for Various Periods, J. Exper. Zool. **19**:99, 1915.

Head, Limbs and Trunk.—In rats underfed from birth to 3 weeks of age, Stewart[3] (1918) found a relative increase of about 16 per cent. in the weight of the head, in comparison with normal controls of the same body weight. In those underfed to 6 or 10 weeks of age, however, the head was nearly normal, the difference not exceeding 4 per cent.

The data from the present series (Table 2) indicate that such rats on refeeding to 25 gm. body weight, or more, rapidly regain their normal relative head weight. It is doubtful whether the slight underweight (—5.8 and —5.7 per cent.) of the head in the first two groups has any special significance.

Jackson[15] found little if any increase in head weight in rats held at maintenance from age of 3 to 10 weeks, and Stewart[2] found nearly normal head weight in such rats throughout the period of refeeding.

Although there is considerable change in the normal proportions of the limbs and trunk of the rat during the postnatal growth period (Jackson and Lowrey[16]), there appears to be no marked or constant change in their relative weight in the young during various periods of underfeeding and refeeding (Jackson,[15] Stewart,[2, 3] 1916, 1918). Likewise, in the present experiments, as shown by Table 2, the proportions of the forelimbs, hindlimbs and trunk remain nearly normal. The apparent differences are comparatively small, and are probably due chiefly to normal variability and experimental error. Allowance must be made for the difficulty in removing the limbs in a uniform manner.

The Integument.—In rats underfed from birth, Stewart[3] (1918) found no appreciable change in the relative weight of the integument at 3 weeks of age, though at 6 and 10 weeks there was a loss of 43 to 48 per cent., compared with normal controls of the same body weight.

In the present series, assuming that the integument was likewise normal in weight in the rats underfed from birth to 3 weeks, it is difficult to account for the apparent loss of 20.1 per cent. on ample refeeding to a body weight of 25 gm. (Table 2, A). In the succeeding groups, the recovery of normal relative weight is apparent. In the rat refed to 75 gm. after underfeeding from birth to 10 weeks (Table 2, F), there is even an apparent overgrowth of the integument (+32.3

---

[16] Jackson, C. M., and Lowrey, L. G.: On the Relative Growth of the Component Parts (Head, Trunk and Extremities) and Systems (Skin, Skeleton, Musculature and Viscera) of the Albino Rat, Anat. Rec. **6**:449, 1912.

per cent.), although this may be merely an accidental variation. It is difficult to remove the integument in a uniform manner, due to the underlying subcutaneous layer and dermal sheet of musculature.

In rats underfed at later periods, beginning at three weeks of age, Jackson[15] found losses of 36 per cent. or more in the integument; and Stewart[2] found that in such rats the integument recovered its normal relative weight after two weeks or more of ample refeeding.

Skeleton.—The term "ligamentous skeleton" is used to designate the fresh skeleton together with the attached cartilages, periosteum and ligaments. The "cartilaginous skeleton" was prepared by immersion of the ligamentous skeleton for about one hour in 1 per cent. aqueous "Gold Dust" solution (a soap mixture) at about 95 C., so as to remove all the fibrous tissues. The cartilaginous skeleton, after rinsing in clean water, was first weighed in the moist condition. Then it was dried for several days in an oven at about 85 C., to constant weight.

As shown by the data of Stewart[3] (1918), in rats underfed from birth the skeleton shows a remarkable persistence of growth. In the ligamentous skeleton, the relative increase at 3, 6 and 10 weeks (compared with normal controls of the same body weight) is 19, 13 and 24 per cent., respectively. In the moist cartilaginous skeleton, the increase is much greater, amounting to 94, 33 and 54 per cent., respectively, at the same periods. In the dry skeleton, the corresponding increases reach the astonishing figures of 166, 46 and 100 per cent. Thus the growth of the skeleton during inanition was accompanied by a great increase in the amount of dry substance, together with other indications of the normal process of skeletal development.

The present data (Table 2) show that on ample refeeding to body weights of from 25 to 75 gm. after such periods of underfeeding, the skeleton in general rapidly recovers its normal proportions. Excepting the first group, the ligamentous skeleton in all cases has nearly normal relative weight. The cartilaginous skeleton, both moist and dry, even shows a definite tendency to lag behind that in normal controls of the same body weight, especially in those underfed to 6 and 10 weeks (excepting the female refed to 75 gm.). The data are not conclusive, as there is some difficulty in securing uniformity of technic in the removal of the skeleton, but they suggest the possibility that the early inanition period, while not preventing the *immediate* growth of the skeleton, may produce an effect which tends to inhibit the skeletal growth at later periods, even on ample refeeding.

Persistent increase in length, indicating continued growth of the skeleton in malnourished human infants and children, has been noted by Variot,[17] Freund,[18] Birk[19] and Hess.[20] A similar persistent skeletal growth during inanition in young animals was observed in calves by Waters[21] (1908), and by Aron[22] in puppies. The first extensive and detailed study of the matter was by Jackson,[15] who found a marked increase in the skeletons of albino rats held at maintenance by underfeeding from 3 to 10 weeks of age. Stewart[2] found that in such rats, on ample refeeding, the skeleton recovers its normal relative weight within two weeks.

Musculature.—In young rats underfed from birth to the age of 3, 6 or 10 weeks, Stewart[3] (1918) found only a slight increase of from 8 to 12 per cent. in the weight of the musculature, compared with normal controls of the same body weight.

The present data (Table 2) indicate that this slight disproportion in the musculature of such rats has disappeared on ample refeeding to 25 gm. or more of body weight. In those refed after underfeeding to 6 and 10 weeks there is, on the contrary, a slight deficit (—5.7 to —15.4 per cent.) in the weight of the musculature. This deficit is of somewhat doubtful significance, but may indicate that the earlier inanition period has a retarding effect on the later growth of the musculature.

In rats held at maintenance by underfeeding from 3 to 10 weeks of age, Jackson[15] found but a very slight increase (3 per cent.) in the weight of the musculature; and Stewart[2] found the musculature of such rats nearly normal in weight after one week of ample refeeding.

Visceral Group and "Remainder."—The visceral group includes all of the individual organs listed in Table 2. As shown by Stewart[3] (1918) the weight of the combined visceral group increases relatively in young rats underfed from birth to 3, 6 or 10 weeks, the increase being 28, 29 and 38 per cent., respectively.

The present data (Table 2) show that in general this excess weight of the visceral group has disappeared in such rats when amply refed to 25, 50 and 75 gm. in body weight. The differences are probably due chiefly to accidental variation. There are, however, differences among the various individual organs, which will be considered later. It should be remembered that the weight of the visceral group is dominated largely by the weight of a few larger organs, such as the brain and alimentary tract.

---

[17] Variot, G.: La dissociation de la croissance dans l'atrophie et l'hypotrophie infantiles; atrophie ponderale, atrophie staturale, Bull. et mém. Soc. méd. d. hôp. de Paris, **24**:1347, 1907. Also in Bull. Soc. de pédiat. de Paris **9**:363, 1907.

[18] Freund, W.: Zur Pathologie des Längenwachstums bei Säuglingen und über das Wachstum debiler Kinder, Jahrb. f. Kinderh. **70**:752, 1909.

[19] Birk, W.: Unterernährung und Längenwachstum beim neugeborenen Kinde, Berl. klin. Wchnschr., J. 48 **2**:1227, 1911.

[20] Hess, Alfred F.: Infantile Scurvy. III. Its Influence on Growth (Length and Weight), Am. J. Dis. Child. **12**:152, 1916.

[21] Waters, H. J.: The Capacity of Animals to Grow Under Adverse Conditions, Proc. 29th Ann. Meeting, Soc. for Promotion of Agric. Sc. 1908, 71-96. Ibid. The Influence of Nutrition on Animal Form, Presented at the Thirtieth Meeting of the Society for the Promotion of Agricultural Science, 1909.

[22] Aron, Hans: Nutrition and Growth, I, Philippine J. Sc., B. **6**:1, 1911.

In rats held at maintenance by underfeeding, beginning at the age of 3 weeks, Jackson[15] found in the visceral group a definite increase at 6 and 8 weeks, less marked at 10 weeks. On amply refeeding such rats, Stewart[2] found that the visceral group recovered normal relative weight within a week.

The "remainder" is obtained by subtracting the weight of the integument, ligamentous skeleton, musculature and viscera from the net body weight (intestinal contents excluded). This "remainder" includes various small, unweighed organs, dissectable fat, body fluids, and loss by evaporation, etc. In rats underfed from birth, Stewart[3] (1918) found a very marked loss in the weight of the remainder at 3 weeks (—40 per cent.), with smaller losses at 6 and 10 weeks (—19 and —23 per cent.).

The present data (Table 2) show that in such rats the loss in the "remainder" has been more than fully recovered on ample refeeding to a body weight of 25 gm. or more, excepting the one female underfed to 10 weeks and refed to 75 gm. The considerable excess in Groups D and E is of uncertain significance, since the "remainder" is notably variable.

In rats held at maintenance by underfeeding from 3 to 6, 8 or 10 weeks of age, Jackson[15] found but little change in the weight of the "remainder"; and Stewart[2] found it about normal after various periods of refeeding.

Brain.—In rats underfed from birth to 3 weeks, Stewart[3] (1918) found a remarkable increase in the weight of the brain, the average (sexes combined), amounting to 60 per cent. gain in comparison with normal controls of the same bodyweight. In rats with continued underfeeding to 6 or 10 weeks of age, the excess in brain weight was much less, being 3 and 8 per cent., respectively.

In contrast with this overweight of the brain in the underfed rats, the present data (Table 2) show a uniform underweight of the brain in rats similarly underfed and subsequently amply refed to a bodyweight of from 25 to 75 gm. In Group D the deficit is small (—4.2 per cent.), but in the other five groups it is considerable (—11.7 to —22.5 per cent.). This indicates clearly that in spite of the persistent growth of the brain during the earlier underfeeding period, it has been in some way affected by the inanition, so that its later growth on ample refeeding is somewhat retarded, at least within the period of the present experiments.

Hatai[23] noted an apparent decrease of 4 or 5 per cent. in the weight of the brain in malnourished young rats with protein-poor diet. In a series similarly underfed and then placed on full normal diet, Hatai[8] found later full recovery in the weight of the body and central nervous system. In another underfed series[24] the brain weight apparently remained normal. Donaldson[25] observed a slight increase of the brain weight in rats held nearly at maintenance by underfeeding from 30 to 51 days of age. Jackson[14] found the brain weight nearly constant in rats held at maintenance by underfeeding from 3 weeks of age for various periods, and Stewart[2] found the brain weight normal (in proportion to body weight) in such rats on ample refeeding for various periods.

In a more recent investigation, Stewart[26] found an excess of 33 per cent. in the brain weight of rats underfed from birth to 3 weeks of age (body weight 10 gm.) and of 30 per cent. in those underfed to 8 weeks (body weight 12 gm.). In another series starved more severely so as to hold them at birth weight for an average of 16 days, a remarkable increase of 114 per cent. in the brain weight was found. Stewart further demonstrated that during its growth in these underfeeding periods the brain shows normal growth in the relative weight of its various parts (excepting perhaps the olfactory bulb).

Variot and Lassablière[27] likewise found the growth of the brain in malnourished human infants to be retarded less than the increase in body weight, the brain thus increasing at the expense of other parts of the body.

Spinal Cord.—In rats underfed from birth, Stewart[3] (1918) found (in comparison with normal controls of the same body weight) a relative increase of 70 per cent. in the weight of the spinal cord at 3 and 10 weeks of age, and of 38 per cent. at six weeks.

---

[23] Hatai, S.: The Effect of Partial Starvation on the Brain of the White Rat, Am. J. Physiol. **12**:116, 1904.

[24] Hatai, S.: Preliminary Note on the Size and Condition of the Central Nervous System in Albino Rats Experimentally Stunted, J. Compar. Neurol. and Psychol. **18**:151, 1908.

[25] Donaldson, H. H.: The Effect of Underfeeding on the Percentage of Water, on the Ether-Alcohol Extract, and on the Medullation in the Central Nervous System of the Albino Rat, J. compar. neurol. **21**:139, 1911.

[26] Stewart, Chester A: Changes in the Weights of the Various Parts, Systems and Organs in Albino Rats Kept at Birthweight for Various Periods, Am. J. Physiol. **48**:67, 1919. Ibid., Weights of Various Parts of the Brain in Normal and Underfed Albino Rats at Different Ages, J. Compar. Neurol. **29**:511, 1918.

[27] Variot and Lassablière: Autonomie du développement de l'encéphale dans les retards de la croissance chez les jeunes enfants, Compt. rend. Soc. de biol. **66**:106, 1909.

The present series (Table 2) show an apparent underweight of the spinal cord in such rats when amply refed to a body weight of 25 to 75 gm. The only exception is in the first group (A), which shows a slight excess of 5.4 per cent. The deficits in the other groups are small and probably insignificant, except in Groups E and F. Here the deficits of 7.5 and 11.2 per cent. suggest that the longer underfeeding period may have an inhibitory after effect on the growth of the spinal cord, similar to that found in the brain.

Donaldson[25] likewise observed an increase in the spinal cord of rats held at maintenance by underfeeding from 30 to 51 days of age, and Hatai[8] found a recovery of normal weight in rats subjected to partial starvation at 1 month of age and then placed on full normal diet. Jackson[15] found an increase of 36 per cent. in the weight of the spinal cord of rats held at maintenance from 3 to 10 weeks of age, and Stewart[2] showed that normal relative weight of the cord was obtained within two weeks of ample refeeding.

Eyeballs.—In rats underfed from birth to 3, 6 or 10 weeks, Stewart[3] (1918) found a relative increase in the weight of the eyeballs (compared with normal controls of the same body weight) amounting to 41, 34 and 66 per cent., respectively.

The present data (Table 2) indicate a small overweight (with one exception) in such rats amply refed to from 25 to 75 gm. in body weight. As the excess in but one case (Group D) exceeds 10 per cent., the interpretation is somewhat doubtful. It is possible that the overgrowth of the eyeballs during inanition may persist somewhat longer than in other organs during the subsequent refeeding period.

A continued growth of the eyeballs was observed by Jackson[15] in rats held at maintenance by underfeeding from 3 weeks of age for various periods. At 10 weeks the excess was 50 per cent. In such rats, Stewart[2] found a recovery of normal weight in the eyeballs after 4 weeks of refeeding.

Hypophysis and Pineal Body.—In rats underfed from birth to 3, 6 or 10 weeks of age, Stewart found in the weight of the hypophysis relative increases of 33, 8 and 24 per cent., respectively. No sexual difference in the weight of the organ appeared under these conditions.

The present data (Table 2) indicate that in such rats amply refed to reach a body weight of from 25 to 75 gm., the hypophysis has returned nearly to normal relative weight, the differences being probably within the range of normal variation and experimental error. There is in Group C a sexual difference in the size of the normal hypophysis, as seen in Groups D and E, where the same control rats are separated according to sex. If in Group C the controls and test rats are grouped separately according to sex, however, the test rats show a decrease in the hypophysis of both sexes, the result being similar to that shown in the table, where the sexes are combined.

In rats held at maintenance by underfeeding from 3 to 10 weeks of age, Jackson[15] noted an increase of 18 or 19 per cent. in the weight of the hypophysis. The normal difference according to sex did not appear. In such rats, on ample refeeding, Stewart[2] found the hypophysis weight somewhat variable, but apparently within the normal range of variation. The volumetric and histologic changes in the hypophysis during inanition and refeeding have been described in detail by Jackson.[28]

The pineal body appears to undergo no constant change in weight in rats underfed from birth to 3, 6 or 10 weeks of age, according to Stewart[3] (1918).

As seen in Table 2, the present data, with one exception (Group D) show in such rats when amply refed to from 25 to 75 gm. body weight a relative loss in the weight of the pineal body, varying from 5.6 to 25.2 per cent. This would seem to indicate that early inanition (especially when prolonged) tends to inhibit the later growth of the pineal body. But the small size of the gland and the relatively few observations makes the conclusion somewhat uncertain.

In rats held at maintenance from 3 to 12 weeks of age, Stewart[2] found an apparent loss in the weight of the pineal body, with recovery after from two to four weeks of refeeding. In rats underfed for longer periods, the results were variable. No sexual difference appears in the weight of the pineal body, as has been found to occur normally in the hypophysis, suprarenal and parathyroid glands (Hatai, Jackson).

Thyroid Gland.—In rats underfed from birth to 3, 6 or 10 weeks of age, Stewart[3] (1918) found no constant change in the relative weight of the thyroid gland.

In the present series (Table 2) there appears to be a slight relative loss (4.0 to 15.6 per cent.) in the rats underfed from birth and refed to 25 or 50 gm. body weight. The three groups refed to 75 gm. are above normal, though in all the differences (4.9 to 7.2 per cent.) are probably within the range of normal variation.

In rats held at maintenance from 3 to 10 weeks of age, Jackson[15] found a marked loss in the weight of the thyroid gland. Stewart[2] concluded that such rats (after maintenance from 3 to 12 weeks of age) on ample refeeding four weeks or more had probably regained normal thyroid weight, although there appeared a slight deficit of

---

28. Jackson, C. M.: Effects of Inanition and Refeeding on the Growth and Structure of the Hypophysis in the Albino Rat, Am. J. Anat. **21**:321, 1917.

6.2 per cent. The histologic changes in the thyroid and parathyroid glands during inanition are described by Jackson.[29]

Thymus.—During inanition in young rats, the thymus undergoes the well-known "hunger involution" which has been described in detail by Hammar and his pupils. In rats underfed from birth to 3, 6 or 10 weeks, Stewart[3] (1918) found losses in the relative weight of the thymus of 30, 60 and 80 per cent., respectively.

The present data (Table 2) are somewhat variable, indicating that in such rats amply refed to body weight of from 25 to 75 gm. the thymus in most cases still remains somewhat below normal weight.

In rats held at maintenance from 3 to 10 weeks of age, Jackson[15] found a loss of 90 per cent. in the weight of the thymus. According to Stewart,[2] this loss is fully recovered after between two and four weeks of ample refeeding. At four weeks of refeeding there was even an apparent "overcompensatory" growth of the thymus, which does not appear in the present series of experiments.

Jonson[30] underfed a rabbit (6 weeks old) for 31 days, the body weight increasing meanwhile from 553 to 655 gm. After ample refeeding for 3 weeks, the weight of the thymus was found equal to that of a normal control killed at 6 weeks of age. Salkind[31] found that at least one week of refeeding is required to restore the normal lymphoid structure of the thymus in (adult) rats after two days of acute inanition.

Heart.—In rats underfed from birth, Stewart[3] (1918) found little change in the weight of the heart at 3 weeks of age, but a relative increase of 13 per cent. at six weeks and of 27 per cent. at ten weeks.

The present data (Table 2) show the heart practically normal in weight in such rats amply refed to 25, 50 and 75 gm. in body weight after underfeeding from birth to 3 or 6 weeks. The apparent increases (19.3 and 13.8 per cent.) on refeeding after underfeeding from birth to 10 weeks are of doubtful significance.

In rats held at maintenance for various periods beginning at the age of 3 weeks, Jackson[15] found the heart nearly normal in weight. On refeeding after maintenance from 3 to 12 weeks of age, Stewart[2] found the heart probably nearly normal in weight, though somewhat above the Wistar norm in the earlier stages of refeeding.

Lungs.—In rats underfed from birth to the age of 3, 6 or 10 weeks, Stewart[3] (1918) found the weight of the lungs about 26 per cent. below that of normal controls with the same body weight.

---

[29] 29. Jackson, C. M.: Effects of Inanition on the Structure of the Thyroid and Parathyroid Glands of the Albino Rat, Am. J. Anat. **19**:305, 1916.

[30] 30. Jonson, A.: Studien über die Thymusinvolution. Die Akzidentelle Involution bei Hunger, Arch. f. mikr. Anat. **73**:390, 1909.

[31] 31. Salkind, J.: Contributions histologiques à la biologie comparée du thymus, Arch. de zool. exper. **55**: Fasc. 5, 1915.

The present data (Table 2) indicate that the lungs in such rats underfed from birth to 3 or 6 weeks and then amply refed to a body weight of from 25 to 75 gm. have usually recovered their normal weight. The apparent overweight in the lungs in those refed after underfeeding to 10 weeks is perhaps due to unrecognized pathologic conditions, which frequently occur in the lungs of the older rats.

In rats held at maintenance by underfeeding from 3 weeks of age for various periods, an average loss of about 15 per cent. in the weight of the lungs was observed by Jackson.[15] Stewart[2] found that in such rats the lungs regained their normal weight within the first weeks of ample refeeding, with an apparent overweight after longer periods of refeeding.

Liver.—In rats underfed from birth to 3, 6 or 10 weeks of age, Stewart[3] (1918) noted increases of 17, 83 and 64 per cent., respectively, in the relative weight of the livers.

The present data (Table 2) are somewhat irregular, but indicate that in such rats amply refed to a body weight of from 25 to 75 gm. the liver weight, as a rule, is probably within the limits of normal variability.

In rats held at maintenance by underfeeding from the age of 3 weeks, Jackson[15] found that the liver tends to gain in weight in the earlier periods, but to lose in weight later. On ample refeeding after maintenance from 3 to 12 weeks of age, Stewart[2] found the liver normal in weight after one week. There was some apparent hypertrophy (probably due to accidental variation) in the rats refed four weeks or more.

Spleen.—In rats underfed from birth to 3 weeks of age, Stewart[3] (1918) found a loss of 49 per cent. in the relative weight of the spleen, while in those underfed from birth to 6 or 10 weeks there was a gain of 31 or 23 per cent., respectively.

The present data (Table 3) indicate that on ample refeeding of such underfed rats there is found an overweight of the spleen at body weight of 25 gm. (52.1 per cent.), and of 50 gm. (33.8 and 112 per cent.); but normal or underweight on refeeding to 75 gm. (—24.3 and —11.4 per cent.). This suggests a tendency to overcompensatory growth on refeeding for a short time, with a tendency to lag behind later.

This idea is supported by results of inanition experiments beginning on somewhat older rats. In rats held at maintenance from 3 weeks of age, Jackson[15] found the spleen nearly normal in weight at 6 weeks, with considerable decrease later (—42 per cent. at 10 weeks). On amply refeeding such rats after maintenance from 3 to 12 weeks of age, Stewart[2] found rapid recovery of the normal weight of the spleen in one-half week, followed by apparent overgrowth (overcompensatory hypertrophy?) in the following two weeks, but with normal weight after four weeks or more of refeeding. Final conclusions must be guarded, however, since in weight the spleen is known to be one of the most variable organs in the body (Jackson[4]).

Stomach and Intestines.—The stomach and intestines are of especial interest in connection with the subject of inanition. They will be considered first in regard to their weight and second in regard to length.

A. Weight of Stomach and Intestines.—In rats underfed from birth to 3, 6 or 10 weeks, Stewart[3] (1918) found increases of 24, 54 and 98 per cent., respectively, in the relative weight of the empty stomach and intestines (including mesentery and pancreas). In the stomach and intestine with contents, there was a loss of 25 per cent. in weight at three weeks, with gains of 104 and 55 per cent. above normal at 6 and 10 weeks, respectively.

The present data (Table 2) indicate that on refeeding such rats to body weight of from 25 to 75 gm., the empty canal apparently remains above normal weight in all except Group D. Those underfed from birth to 3 weeks have become practically normal at body weight of 75 gm., however. The others may require a longer time to reach the normal proportions. The canal with contents appears in general more nearly normal in weight.

In rats held at maintenance from 3 weeks to 6, 8 or 10 weeks of age, Jackson[15] found the empty stomach and intestines increased in weight 60, 48, and 28 per cent., respectively. Somewhat similar increase was also found in the canal with contents, which are watery or mucous in character in the rats during inanition. On refeeding after maintenance from 3 to 12 weeks of age, Stewart[2] found the tract, both empty and with contents, usually returned to nearly normal weight after two weeks.

B. Length of Intestines.—The growth changes previously described in the weight of the alimentary canal during inanition might be due to changes in length, in caliber or in thickness of the wall. Some measurements on the lengths of the large and small intestine have therefore been made by Stewart, and the average data are recorded in Table 3. The intestines were measured in an extended condition, after being slit open for the entire length, the contents and mesentery being removed. In addition to the rats listed in Table 1, some observations on other individuals, used in other investigations by Stewart[3] (1918 and 1919) are included.

Unfortunately, the length of the stomach was not recorded, so the canal as measured is not strictly comparable with the weight given in Table 2. The approximate weight of the intestines (plus mesentery), however, may be estimated by subtracting the corresponding weight of the stomach and pancreas, according to the data of Hatai.[32] The result indicates that the curve of growth in the weight of the intestines alone is somewhat similar to that for the entire tract. The weight of the intestines apparently increases much more rapidly than the body weight, its relative (percentage) weight increasing about fourfold between birth and the age of 6 weeks. In the meantime, as shown by the data in Table 3, the length of the intestines has also increased more rapidly than the body length, their ratio increasing from about 4.3 at birth to 7.3 at 49 days of age. This relative increase in the length of the intestine is insufficient alone to account for its increase in weight, however, hence there must also be a certain amount of increase in its caliber or in thickness of the wall, or both.

As to relative lengths of the large and small intestine (Table 3, A), in all except the new-born group the ratio remains fairly constant between 7.4 and 7.8. This would indicate somewhat uniform rate of growth in length in both large and small intestines.

In the groups underfed from birth (Table 3, B), there is a marked increase in the lengths of both large and small intestines, which is especially marked in those held at birth weight for 19 days and those underfed to 10 weeks of age. This agrees with the increased weight of the tract, observed by Stewart[3] (1918 and 1919). In the group held at birth weight, the relative increase in length appears greater in the large intestine (+81 per cent.) than in the small (+25 per cent.). In the remaining groups, the increase appears to affect both large and small intestines more nearly in equal proportion.

In the rats amply refed after underfeeding from birth to 3, 6 and 10 weeks of age, the intestines in general still appear relatively long (compared with normal controls of the same body weight) in the rats refed to body weight of 25 gm. In those refed to 50 gm. the intestinal length is usually a little above normal, and in those refed to 75 gm., a little below normal. Excepting the latter group, the changes in length of the intestines in the refed groups correspond in general to the changes in weight of the canal (including stomach) previously shown. There is not much change in the relative lengths of the large and small intestines, as indicated by their ratios (Table 3).

---

32. Hatai, S.: On the Weight of the Epididymis, Pancreas, Stomach and of the Submaxillary Glands of the Albino Rat (*Mus norvegicus albinus*) According to Body Weight, Am. J. Anat. **24**:71, 1918.

Suprarenal Glands.—In rats underfed from birth to 3 or 6 weeks, Stewart[3] (1918) found a relative increase of about 60 per cent. in the weight of the suprarenals, while in rats underfed to 10 weeks the increase reached 114 per cent.

The present data (Table 2) indicate that in such rats amply refed to 25, 50 and 75 gm. body weight the suprarenals have returned nearly to normal proportions. Groups B and C show essentially the same result when the data are treated separately according to sex, so they are combined in the table for economy of space. Group A is below the weight at which a sexual difference in the weight of the suprarenal appears.

In rats held at maintenance from 3 weeks of age, Jackson[15] found a slight increase in the weight of the suprarenals at 6 and 8 weeks, with a marked increase at 10 weeks (39 per cent. in males, 12 per cent. in females). On refeeding such rats fully after maintenance from 3 to 12 weeks, the suprarenals appeared slightly below the controls in weight during the first four weeks, and normal thereafter. The volumetric and histologic changes in the suprarenal during inanition and refeeding are discussed in detail by Jackson.[33]

Kidneys.—In rats underfed from birth to 3, 6 or 10 weeks of age, Stewart[3] (1918) finds in the kidneys increases in relative weight amounting to 21, 45 and 38 per cent., respectively.

The present data (Table 2) indicate that in such rats amply refed to a body weight of 25 to 75 gm. the kidneys have in general returned to nearly normal proportionate weight. In Groups E and F, the kidneys are still above normal (17.9 and 14.7 per cent., respectively), possibly because sufficient time has not yet elapsed to allow full recovery of the normal proportions.

In rats held at maintenance from 3 weeks of age, Jackson[15] found a definite tendency to increase in weight of the kidneys at 6 and 8 weeks, with but slight increase (+4.1 per cent.) at 10 weeks. On refeeding fully after maintenance from 3 to 12 weeks of age, Stewart[2] found the kidneys normal in weight throughout the various periods.

Testes and Epididymides.—In rats underfed from birth to 3, 6 and 10 weeks of age, Stewart[3] (1918) found the testes 188, 62 and 51 per cent., respectively, above normal weight. The epididymides showed corresponding gains of 95 and 13 per cent. at 3 and 6 weeks, with a loss of 6 per cent. at 10 weeks of age.

The present data (Table 2) indicate that in such rats the relative overweight of the testes at the end of the underfeeding period apparently persists to a certain extent (+17.5 and 19.2 per cent.) in the rats amply refed to a body weight of 25 or 50 gm. In those refed to 75 gm., the marked loss (—44.6 and —49.4 per cent.) suggests that possibly the earlier inanition in some way affects unfavorably the later growth of the testes. The effects on the epididymides are not so marked, but they also show subnormal weight in the groups refed to a body weight of 75 gm.

---

[33] Jackson, C. M.: The Postnatal Development of the Suprarenal Gland and the Effects of Inanition on Its Growth and Structure in the Albino Rat, Am. J. Anat. **25**: No. 3, 1919.

In rats held at maintenance from 3 to 10 weeks of age, Jackson[15] found an increase of 34 per cent. in the weight of the testes, with uncertain results in the epididymides. On refeeding after maintenance from 3 to 12 weeks of age, Stewart[2] obtained somewhat variable results; the testes and epididymides appeared somewhat below normal in the earlier refeeding periods, but normal later.

Ovaries.—In rats underfed from birth to 3, 6 and 10 weeks of age, Stewart[3] (1918) found relative increases in the weight of the ovaries amounting to 83, 91 and 54 per cent., respectively.

The present data (Table 2) for the ovaries in such rats amply refed to a body weight of 25, 50 or 75 gm. are somewhat variable and inconclusive. Those refed after underfeeding to 3 weeks show a moderate overweight (+22.3 and +15.8 per cent.) at 25 and 75 gm., but a slight loss (—3.7 per cent.) at 50 gm. Those refed after underfeeding to 10 weeks show a marked loss (—20.4 and —28.6 per cent.), suggesting that the prolonged inanition period may affect unfavorably the later growth of the ovary.

In rats held at maintenance from 3 weeks of age, Jackson[15] found a slight gain in the ovaries at 6 weeks, but a marked loss (—27 per cent.) at 10 weeks. In rats refed after maintenance from 3 to 12 weeks of age, Stewart[2] found a tendency to overweight of the ovaries in the earlier refeeding periods, normal after four weeks, and underweight in adults. Conclusions are difficult on account of the large variation in the weight of the ovaries due to their normal functional cycles, but the evidence indicates that early inanition may permanently affect the later growth of the ovaries, even though the unfavorable effects may apparently disappear for a time during the earlier periods of refeeding.

DISCUSSION

From previous investigations (cf. summary by Jackson and Stewart[1] (1918), it is known that the growth of the various organs and parts in underfed young rats varies greatly, not only in different organs, but in the same organ according to the age of the animal and the extent and severity of the inanition period. Organs which in early and shorter periods of inanition increase considerably in weight may later barely maintain themselves or may even lose weight. In general, as age advances, the growth reactions in organ weights during inanition tend to approach those found during inanition in adults. Generally speaking, the growth reaction of an organ in a young animal during inanition will vary according to the intensity of normal growth in that organ at a corresponding normal body weight, but there are numerous exceptions to this rule. The most striking growth reactions in many of the organs are found in rats held at birth weight for two or three weeks, a period when the normal growth rate in general is most rapid (Stewart,[3] 1919).

Since the normal growth rate and the growth during inanition vary so greatly according to age and other factors in the various organs, it would naturally be expected that the rate and extent of recovery on ample refeeding might likewise vary according to circumstances. The evidence at present available demonstrates that this is the case in young rats refed after different periods of inanition. We have seen in the present series of experiments that the results in some organs vary, not only according to the length of the refeeding period (whether to 25, 50 or 75 gm. body weight), but also according to the length of the preceding inanition period.

That the process of recovery on refeeding will also vary according to the age at which the preceding inanition period began is evident if the results of the present experiments (beginning at birth) are compared with those published earlier by Stewart[3] (1918), in which the rats were amply refed after maintenance by underfeeding beginning at 3 weeks of age. In the rats in which the inanition tests began at the later age, most of the organs (excepting the testes, epididymides and suprarenals, which remained subnormal) had recovered approximately their normal relative weight within from two to four weeks of refeeding. The thymus, and possibly the lungs, spleen and ovaries, were apparently even above normal (overcompensatory growth?) after from two to four weeks of refeeding. In the present experiments, in which the underfeeding began at birth, we find somewhat different results. In general, inanition at the earlier age seems to have effected more permanent results, as reflected in the more tardy recovery of normal weight in several of the organs. The suprarenals, it is true, do not appear subnormal; but to the testes and epididymides we must add (for the present series) the cartilaginous and the dry skeleton, brain, spinal cord, thymus, and perhaps the pineal body, thyroid gland and ovaries, as showing more or less retarded recovery in weight on refeeding. There is no evidence of overcompensatory growth in the thymus and ovaries of the present series, and the overweight of the lungs in both cases is perhaps abnormal. In the present series, however, there usually appears a distinct hypertrophy of the intestinal canal, and possibly of the eyeballs. The possibility of an early overcompensatory growth in the spleen as well as the testes, followed by later retardation in growth, is also recognized.

There is some difficulty in making direct comparison between the present series and that of Stewart,[2] as they differ more or less in the length of the preceding inanition and subsequent refeeding periods, as well as in the age at which the experiments began. Moreover, it must always be remembered that the number of observations is too small for anything but provisional conclusions. More extensive data will doubtless modify the results to some extent. The final effects on the various organs in the rats underfed at different periods and later refed to their maximum size (adult) will be considered in a separate article.

SUMMARY

From the data in the present series of refeeding experiments, the following provisional conclusions are indicated:

1. In albino rats underfed from birth to 3, 6 or 10 weeks of age, rapid growth in body weight ensues on ample refeeding. Body weights of from 25 to 75 gm. are apparently reached more rapidly in those refed after underfeeding to 10 weeks of age than in those refed after underfeeding for shorter periods.

2. The body length remains slightly above normal in the group refed to 25 gm. body weight, but appears nearly normal in the others. The tail length and the weights of the head, limbs and trunk appear nearly normal in all the refed groups.

3. As to the body systems, the integument appears subnormal in weight in the group refed to 25 gm., usually normal (or above) in the others. The ligamentous skeleton appears nearly normal in all refed groups, but the cartilaginous skeleton (moist or dry) tends to be subnormal in weight. The musculature is nearly normal in weight, with a slight apparent deficit in the later refeeding periods. The visceral group (as a whole) and the "remainder" show no constant or significant variations in the refed groups.

4. The individual organs differ greatly in the extent to which they have recovered their normal weight (compared with that in controls of the same body weight) in the various groups refed to a body weight of 25, 50 or 75 gm. after underfeeding from birth to 3, 6 or 10 weeks of age. The weights of the various organs in the refed rats are as follows.

The hypophysis and suprarenal glands are apparently nearly normal in weight in all groups; likewise the heart, lungs and kidneys, excepting an apparent overweight (of doubtful significance) in the groups refed after underfeeding to 10 weeks of age. The liver is rather irregular in weight, but probably within the range of normal variation.

The brain, spinal cord and thymus appear almost constantly subnormal in weight in all test groups. The apparent loss in the pineal body is of somewhat uncertain significance. The thyroid gland appears subnormal on refeeding to 25 and 50 gm. after underfeeding to three weeks; otherwise normal. The ovaries are irregular in weight, apparently subnormal in those refed after underfeeding to 10 weeks. The epididymides appear subnormal on refeeding to body weight of 75 gm.

The empty stomach and intestines are usually above normal weight in the refed groups; the canal with contents appears more nearly normal in weight. The length of the intestines is somewhat above normal on refeeding to 25 gm. body weight; nearly normal later. There is little change in the relative lengths of large and small intestines. The eyeballs show a slight overweight, of doubtful significance.

Two organs show an apparent tendency to overcompensatory growth in the earlier stages of refeeding, with later retardation. The spleen is greatly above normal weight at body weight of 25 or 50 gm.; but normal or subnormal at body weight of 75 gm. The testes show a similar reaction, the apparent atrophy in the later stages of refeeding being especially marked.

46.7.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JUNE 2

Reprinted from THE AMERICAN JOURNAL OF
ANATOMY, Vol. 25, No. 4, July, 1919

STUDIES ON THE MAMMARY GLAND

IV. THE HISTOLOGY OF THE MAMMARY GLAND IN MALE AND FEMALE ALBINO RATS FROM BIRTH TO TEN WEEKS OF AGE

J. A. MYERS

Institute of Anatomy, University of Minnesota, Minneapolis

NINETEEN FIGURES (ELEVEN TEXT FIGURES, TWO PLATES)

In earlier works (Myers, '16 and '17 b) the growth and distribution of the milk-ducts in male and female albino rats from birth to ten weeks of age were studied. Owing to the fact that the finer structure of the gland during these ages has not been carefully studied and that a knowledge of such structure is essential before proceeding with the changes in the gland during pregnancy, the present work was undertaken. An abstract of the results has already been published (Myers, '18).

MATERIAL AND TECHNIQUE

In this work approximately forty apparently healthy rats of about normal weight were used. The age ranged from birth to ten weeks. These individuals were fairly evenly distributed among the described stages. For general histological work the glands were fixed in Zenker's fluid, paraffin sections cut 7 or 8 μ and stained with iron-hematoxylin or Mallory's anilin-blue connective-tissue stain. Other glands were stained with Dominici's combination stain. For the study of elastic fibers, glands were fixed in 10 per cent formalin and stained in Weigert's resorsin-fuchsin stain or Unna's orcein stain. Mayer's mucicarmine was also applied to some glands with the hope of determining more accurately the nature of the contents of the intraepidermal portion of the primary duct.

The method described by Lane-Claypon and Starling ('06) was used in making cleared preparations.

Born's method was used in making the wax reconstructions.

For the study of the secretion in the new-born, animals were killed by bleeding, and fresh glands were cut with the freezing microtome. Others were fixed in 10 per cent formalin for an hour or so, after which frozen sections were cut. Frozen sections were stained in 1 per cent osmic acid, plain scarlet red (prepared by adding an excess of the stain to boiling 70 or 80 per cent alcohol) or Herxheimer's alkaline scarlet red. Other glands were fixed in Flemming's fluid, paraffin sections cut at 5 μ and stained with safranin. Still others were fixed, cut, and stained according to the method described by Bell ('14).

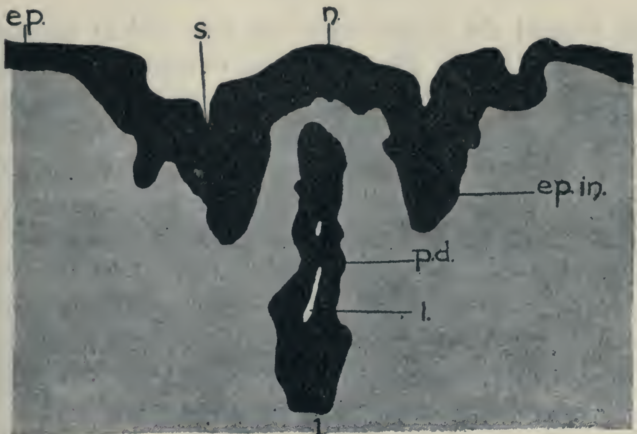
OBSERVATIONS

New-born

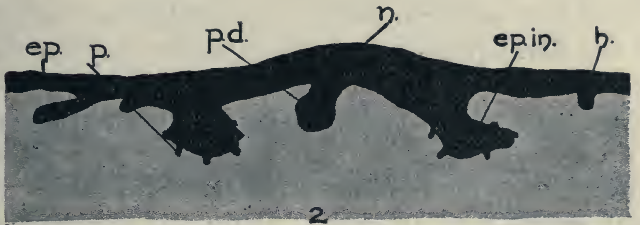
Microscopic sections show that the epidermis over each nipple area of the new-born female albino rat is nearly two times as thick as that in the skin adjacent to this area (fig. 1). Such a thickening renders the skin more opaque, thus preventing the blood in the deeper layers from giving a red appearance to the surface. Therefore, the nipple area appears much lighter than the immediately surrounding skin.

On the surface in the central portion of the light area is a very slight elevation which represents the developing nipple, while partially surrounding the nipple is a shallow depression (fig. 1) which in most cases is partly filled with cornified cells. Peripheral to the depression the epidermis becomes continuous as a much thinner layer surrounding the mammary gland area.

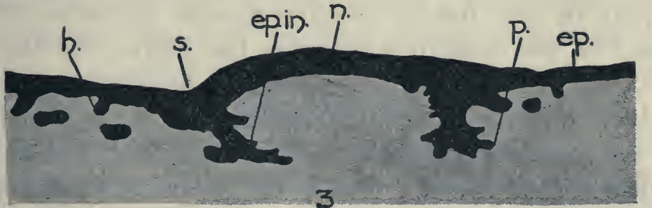
Deep to the depression is the epithelial hood described in earlier papers (Myers, '16, '17 a, and '17 b). In the new-born the free projection of the hood extends slightly further into the corium than in twenty-day fetuses. The outer and inner surfaces of this projection are smooth and are covered with the stratum germinativum. The stratum corneum dips slightly between the two surfaces of the hood.



1



2



3

Fig. 1. Drawn from a section through the left first inguinal nipple of a female albino rat at birth. *ep.*, epidermis adjacent to mammary gland area; *ep. in.*, epithelial ingrowth or hood; *1.*, lumen; *n.*, nipple; *p.d.*, primary duct. × 100.

Fig. 2. Drawn from a section through the left second thoracic nipple of a female albino rat of one day. *ep.*, epidermis adjacent to mammary gland; *ep. in.*, epithelial ingrowth; *h.*, hair follicle; *n.*, nipple; *p.*, developing processes on epithelial hood; *p.d.*, primary duct. × 100.

Fig. 3. Drawn from a section through the right third thoracic nipple of a female albino rat of five days. *ep.*, epidermis adjacent to mammary gland; *ep. in.*, epithelial ingrowth; *h.*, hair follicle; *n.*, nipple; *p.*, developing processes on epithelial hood; *s.*, sulcus partially surrounding nipple. × 100.

Traced toward the nipple from the free end of the epithelial projection the stratum germinativum extends toward the surface of the skin thus lining the inner surface of the epithelial hood. Traced away from the nipple from the free end of the epithelial projection it forms the outer surface of the projection and becomes continuous as the stratum germinativum of the surrounding integument. Mitotic figures are numerous in the stratum germinativum covering the epithelial projections.

The contents of the hood are densely arranged connective-tissue cells, small blood-vessels, small nerves, white blood corpuscles, and the developing primary milk-duct. The connective-tissue cells are densely placed as in other parts of the corium. Near the walls of the epithelial hood these cells are arranged somewhat parallel to the stratum germinativum. In other parts of the hood they are arranged in a more irregular manner except around the developing primary duct, where they are concentrically placed, thus forming the sheath of the duct.

In microscopic sections near the highest part of the developing nipple a very shallow pit is being formed by the process of desquamation. This pit represents the future opening of the primary milk-duct—the milk-pore. As this pit deepens in the later stages it becomes a part of the lumen of the primary milk-duct. This part of the lumen becomes greater in extent than the thickness of the epidermis covering the nipple, for the sake of convenience in description, however, it shall hereafter in this paper be known as the intra-epidermal part of the primary duct. From this very shallow pit a solid cord of cells extends through the thickened epidermis. This is the anlage of the intra-epidermal portion of the primary milk-duct. At birth there is no trace of a lumen in this portion of the duct. That part of the duct within the epithelial hood takes a course perpendicular to the surface of the integument. Near the surface the duct is represented by a solid cord of cells, but as it is traced deeper an occasional lacuna representing the first trace of a lumen is encountered. In some glands several lacunae have flown together, thus representing a larger lumen with an irregular outline (fig. 1). The cells in the walls of this part of the duct present no definite arrangement into layers (fig. 7). As the primary duct leaves the epithelial hood and passes into the tela subcutanea, nearly to its bifurcation, the lumen presents a very irregular outline, in some sections being absent entirely. The walls of the duct are from three to four cells thick.

The secondary ducts which result from the bifurcation of the primary duct possess lumina with more regular outlines. The cell walls appear somewhat thinner and there are indications of the cells beginning to arrange themselves into layers. From the secondary ducts to the ends of the terminal ducts there is, in most cases, a fairly definite layer of low columnar or cuboidal cells surrounding the lumen. External to this layer is a second layer of somewhat irregular-shaped cells which is present in most places, but may be partly or entirely lacking in some parts of the terminal ducts. The free ends of the terminal end-buds are composed of solid masses of epithelial cells.

The milk-ducts are usually spread out so as to occupy a single plane (fig. 18) except in the case of the second inguinal gland (Myers, '16), where the limited space for the distribution of ducts forces them into two or more planes. Immediately surrounding all segments of the ducts are a few concentrically placed layers of connective-tissue which represents the future mantle layer. The irregularly arranged connective-tissue between the adjacent ducts and the mantle layer may be regarded as representing a part of the true stroma found in later stages.

As previously shown (Myers, '17 b), no trace of a mammary gland appears on the surface of the skin of new-born male rats. This is due to the fact that the epidermis in most cases presents no thickening in the region of the mammary gland. The skin, therefore, is as transparent over this region as in that immediately surrounding the gland, so the color of the blood in the corium gives a uniform appearance to both areas.

The end of the primary duct attached to the epidermis in the male is represented by a solid cord of cells. The free end of this duct and the secondary ducts possess lumina in slightly more advanced stages of development than in females of corresponding age. The remaining parts of the gland correspond closely in structure and development with the glands of the female.

Twelve hours

In female rats twelve hours after birth the epidermis covering the developing nipple appears slightly thinner than at birth. There is no noticeable change in the epithelial hood. The attached end of the primary duct is still without a lumen. The remaining ducts possess quite well-developed lumina and their walls are generally lined with two layers of epithelial cells, as described in the preceding stage. However, in some cases the outermost layer is almost entirely absent from a small part of the ducts. These parts with a single layer of cells constituting their epithelial lining are not confined to the terminal ducts only, but may be found in any of the other ducts except the primary duct.

There is a considerable extravasation of red blood-cells in the stroma around the ducts in some specimens. A considerable number of such cells are present in the walls of the milk-ducts, while a few appear in the lumina.

In addition to some red blood-cells in the lumina of the ducts, a few leucocytes are present. A somewhat homogeneous substance is also present in some segments of the ducts. In no case have degenerating cells and nuclei been observed in the lumina of the milk-ducts. The cells and other contents present exist in such small quantities that distention appears in no part of any system of ducts examined. Special fat stains failed to reveal any trace of fat in the walls or lumina of milk-ducts. In the fetus it was found (Myers, '17 a) that the lumina of the milk-ducts are formed by the process of rearrangement of the cells and not, as was formerly believed, by the degeneration of the central cells of the ducts. At birth and for some time after no degenerating cells have been observed in the lumina; moreover, the free borders of the cells immediately surrounding the lumina are quite sharply indicated. It therefore is evident that the substance contained in the lumina of the milk-ducts outside of normal cells is not a detritus, but is probably a secretion produced by the cells surrounding the lumen.

The stroma very distinctly presents the anlages of its two parts, the mantle layer immediately surrounding the ducts and the true stroma intervening between the ducts of a system of ducts. The true stroma is rather compact and contains many developing fat cells located toward the free ends of the ducts.

Twenty-four hours

Twenty-four hours after birth the developing nipple presents about the same appearance as in the preceding stage. The epithelial projection of the hood now possesses a few very short processes, some of which are located on the inner surface and extend toward the primary duct, while others are on the outer surface projecting away from the hood (fig. 2). Such processes are formed by a thickening of the stratum germinativum. In some cases the cells of this layer have only elongated, while in others at least two layers of cells enter into the formation of the processes.

The contents of the lumina are approximately the same in quality and quantity as in the last-described stage. In the walls of the ducts just outside the lumen an occasional vacuole is seen.

In the inguinal region laterodorsal to the inguinal and abdominal glands a long mass of fat is beginning to appear in the tela subcutanea. This mass extends from the lateral surface of the abdomen just anterior to the ilium to a position posterolateral to the vagina. In the remainder of the present paper this mass of fat will be designated as the inguinal fat pad. Adipose tissue is also developing rapidly laterodorsal to the second and third thoracic glands and cephalic to the first thoracic gland, but it does not present such a definite mass as in the inguinal region.

Four and five days

In female rats of four and five days the nipple is somewhat more elevated above the surface of the surrounding skin than at the end of the first day of life (figs. 3 and 12). The nipple is now represented by a somewhat oblong elevation (fig. 12). Starting at a point slightly posterior to the first thoracic nipple and passing cephalad, we find a very gradual elevation until the highest part of the nipple is reached. If we start a short distance cephalic to the nipple, we enter a shallow groove or sulcus before reaching the base of the nipple. From the bottom of this groove we find a steep approach to the summit of the nipple elevation. The groove appears cephalic to and passes slightly to the medial and lateral sides of the nipple. In the case of the second and third thoracic glands, the abdominal gland and the first inguinal gland, the groove is on the medial side and extends slightly to the cephalic and caudal parts of the base of the nipple (fig. 5). It is absent on the lateral side of the nipple. In the second inguinal nipple the relations of the groove to the nipple are just opposite to those in the first thoracic. Near the highest part of the nipple the lumen of the intra-epidermal portion of the primary duct appears somewhat deeper than when it was observed in an earlier stage.

The projection of the epithelial hood extends more deeply into the corium than in the earlier stages (fig. 3). The processes which appeared on the epithelial projection in rats of one day are now longer and have the appearance of rather sharp spines. A few of them possess secondary processes.

The lumen of the primary duct now extends nearer to the surface, yet it is not in continuity with the lumen of the intra-epidermal part of the duct. No degenerating cells have been observed in the formation of the lumen of the primary duct proper. This lumen apparently is formed by a process of rearrangement of cells as was shown to be the case (Myers, '17 a) in the other milk-ducts of the fetus. From the secondary ducts through the terminal ducts the amount of secretion present in lumina has increased somewhat since the end of the first day. While it is present in considerable quantities, yet the ducts are by no means engorged. In sections stained with Weigert's iron-haematoxylin the secretion fails to take the stain, while in those stained with Dominici's mixture it takes a light pink color. In fresh frozen sections very rarely a fat droplet was observed in the walls of the milk-ducts. The infrequency of such droplets, their relation to the cells, and their absence in sections fixed, sectioned in paraffin, and stained in other fat stains, proves fairly conclusively that they were carried by the razor from the fat in the connective-tissue stroma to the epithelial walls of the ducts. Leucocytes inside the lumina are very rare at this stage. A few normal cells with nuclei resembling those of the epithelial wall are present in the lumina. In a few instances normal nuclei with apparently no accompanying cytoplasm were observed in the lumina. Such elements are slightly more numerous toward the free ends of a system of ducts. In only one or two instances have the cells within the lumina shown any signs of degeneration.

Dominici's combination stain reveals some leucocytes in the stroma outside the ducts and in the epithelial hood, but no infiltration of such cells in the stroma around the ducts has been observed. There apparently is no extravasation of red blood-cells as was observed in rats of one day.

End of first week.

At the end of the first week the nipple is more elevated, the lumen of the intra-epidermal part of the primary duct and the projection of the epithelial hood extends deeper into the corium. The lumen of this part of the primary duct is now nearly filled with debris in which appear some desquamated epithelial cells. The processes on the epithelial projection of the hood are very conspicuous.

The milk-ducts in most places possess two layers of epithelium. The inner layer is formed of very compact epithelial cells of the low columnar or cuboid type. The cells of the outer layer are less compact and in fact may be absent in some places. They are irregular in size and shape. The amount and quality of the secretion in the lumina is approximately the same as at four and five days.

At this stage the primary duct of the male rat is attached to the epidermis, there being no nipple at any stage. Its attached end does not possess a lumen. In some cases there is a very slight excavation on the surface of the skin immediately over the attached end of the primary duct which resembles the developing milk-pore. As the primary duct is traced toward the tela subcutanea a distinct lumen appears and the wall is seen to be composed of two layers of cells which are similar in shape and arrangement to those of the walls of ducts of corresponding thickness in the female. The remaining ducts in the male resemble in number, distribution and structure the corresponding ducts in the female. The secretion in the lumina appears the same in every respect as in the female except that it appears present in slightly larger quantities in the male.

In both sexes the free ends of the milk-ducts of the abdominal and inguinal glands are imbedded in the medial margin of the inguinal fat pad, but in most cases they have not penetrated this pad very deeply. Besides the ducts growing toward the inguinal fat pad, the pad itself has increased in size.

Two weeks

It has been pointed out by Myers ('16) that the nipple (in the female rat) takes on a very rapid development during the second week. The present study shows that the greater part of that development occurs in the latter part of the second week. The nipple becomes so large that it is very conspicuous to the naked eye in living animals and has been used to determine the sex at this age (Jackson, '12). Microscopic sections and wax reconstructions (fig. 13) show that the nipple is much more elevated than in the preceding stage. It is at this time a somewhat elongated structure and has not yet taken on the typical form of a nipple. The shallow groove which was present around a part of the base of the nipple in earlier stages is now somewhat deeper. This groove now entirely surrounds the base of the first thoracic nipple, except on the caudal side where the base of the nipple becomes continuous with the surface of the surrounding skin without a groove. In case of the second and third thoracic glands, the abdominal gland, and the first inguinal gland, the groove extends around the base of the nipple except on the lateral side. In the second inguinal gland the groove is absent on the cephalic side. From the bottom of this groove, which lies immediately superficial to the attached end of the epithelial hood, may be seen short strands of epithelial tissue which react to the stains differently from the surrounding epithelium. Higher magnification shows that the cells in such strands are undergoing degeneration and are being cast out, thus deepening the groove.

The epithelial projection of the hood has taken on a marked development since the fifth day. It still presents processes which are now more numerous on the inner side of the projection. Such processes are likewise present on the inner surface of the epidermis covering the nipple.

As the layer of smooth muscle which lies immediately below the hair follicles reaches the neighborhood of the nipple, it turns toward the surface and enters the epithelial hood. Here many of the fibers take a course parallel with the primary duct and apparently enter into the formation of the sheath of the duct. Others soon take an oblique direction and apparently attach to the processes on the inner surface of the epithelial projection. Some muscle fibers, after extending into the nipple, take an oblique course and appear to attach to the processes on the inner aspect of the surface epidermis of the nipple. The function of the smooth muscle fibers will be dealt with in studies on the activity of the mammary gland.

The lumen of the intra-epidermal part of the primary duct contain debris apparently in sufficient amount to distend the walls of this part of the duct. Near the milk-pore the wall is reduced to a rather thin structure. As the bottom is approached, the wall becomes thicker. It is here that desquamation is taking place at present. The cord of epithelial cells, which in all of the previous stages separated the lumen of the intra-epidermal part of the duct from the lumen of the primary duct proper, is at this age perforated by a very narrow canal. Thus for the first time a communication is established between the lumina of the system of milk-ducts and the exterior.

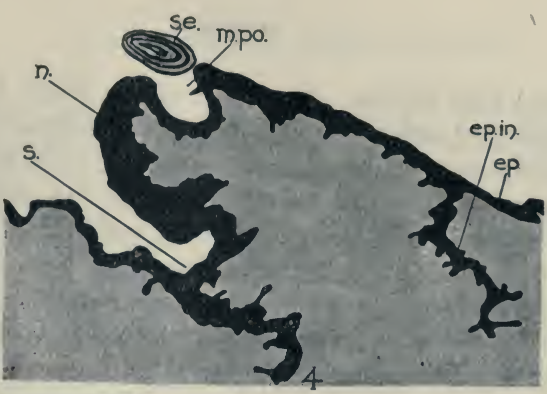
The lumen of that part of the primary duct within the epithelial hood and nipple now has a fairly regular outline as far as the region intervening between it and the luman of the intra-epidermal part of the duct. Here the walls consist of cells (three or four deep) that have not yet arranged themselves into definite layers. As the primary duct is traced from the epithelial hood into the tela subcutanea, where it turns at right angles and courses parallel with the surface of the skin, the walls of the duct become thinner. The cells are here arranged in layers, rarely more than two layers present. The lumen has a well-defined outline which is occasionally interrupted by the budding off of short lateral buds. Many of these buds likewise possess lumina. The remainder of the ducts has the same general appearance in structure as at the end of one week. The epithelial walls are composed of two layers of cells (fig. 8).

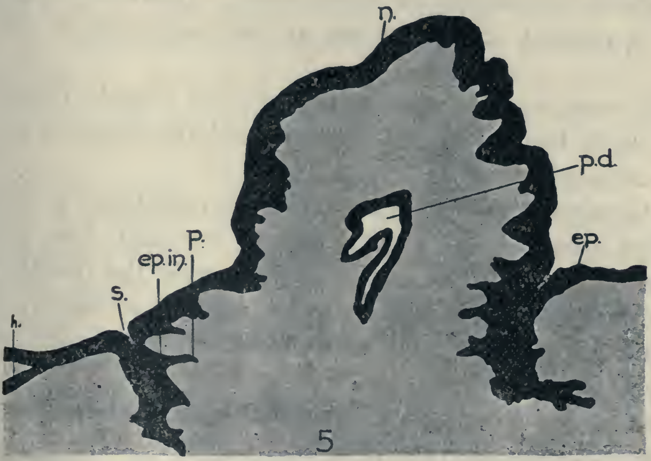
The contents of the milk-ducts have diminished in quantity since the earlier-described stages.

Hair follicles are very numerous peripheral to the sulcus surrounding the nipple superficial to the epithelial projection, but they have in no specimen been observed medial to this groove.

Three, four, five, six, and seven weeks

From the end of the second to the beginning of the eighth week the growth of the mammary gland, as shown in an earlier work (Myers, '16), in the female albino rat is apparently only sufficient to keep pace with the general body growth. Cleared preparations show that beyond question the ducts of the mammary gland grow in length and, moreover, send out numerous new collateral branches during this period. The nipple makes a gradual increase in height. The groove has approximately the same extent around the nipple as in rats of two weeks, but it becomes deeper as age advances. The projection of the epithelial hood retains its processes (fig. 4). The epithelium between the lumen of the intra-epidermal part of the primary duct and the lumen of the primary duct proper at the end of the second week is completely broken down about the fifth or sixth week. The lumen of the primary duct proper therefore becomes directly continuous with that of the intra-epidermal part of the duct and communicates with the exterior through the milk-pore. The lumen of the intra-epidermal portion of the duct is through the seventh week





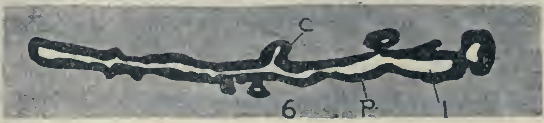


Fig. 4. Drawn from a section through the right second inguinal nipple of a female albino rat of six weeks. *ep.*, epidermis adjacent to mammary gland area; *ep.in.*, epithelial ingrowth; *m.po.*, milk-pore; *n.*, nipple; *s.*, sulcus surrounding a part of base of nipple; *se.*, secretion apparently solidified and dislodged from milk-pore. × 66–$\frac{2}{3}$.

Fig. 5. Drawn from a section through the right first inguinal nipple of a female albino rat of nine weeks. *ep.*, epidermis; *ep.in.*, epithelial ingrowth; *h.*, hair follicle; *n.*, nipple; *p.*, process on epithelial ingrowth; *p.d.*, primary duct; *s.*, sulcus surrounding a part of base of nipple. At the bottom of this sulcus may be seen indications of degeneration. × 66–$\frac{2}{3}$.

Fig. 6. Drawn from a longitudinal section through the primary duct of the right first inguinal gland of a female albino rat of nine weeks. *c.*, collateral duct; *l.*, lumen; *p.*, primary duct proper. × 66$\frac{2}{3}$.

still engorged with debris. That part of the debris which occupies the milk-pore and is in contact with the exterior apparently becomes solid, thus forming a plug. In some specimens this plug has been removed from the milk-pore during the process of sectioning and lies as a solid body outside of, and independent of the milk-pore (fig. 4). Owing to the presence of such a plug, the contents formed in the lumen of the intra-epidermal part of the primary duct are unable to escape.

The contents of the milk-ducts gradually diminish in quantity after about the second or third week. However, considerable debris is present in the lumina at the beginning of the eighth week.

In all specimens examined the smooth muscle fibers of the nipple have the same arrangement and distribution as in the earlier described stages.

The terminal end-buds of the ducts are prominent in the above-described stages. The lumen of the terminal duct gradually increases in size until it reaches a point near the middle part of the end-bud (fig. 11). Here the lumen ends abruptly, and the remainder of the end-bud is represented by a solid mass of epidermal cells.

It has been pointed out (Myers, '17 b) that the milk-ducts of the male fail to proliferate as rapidly as those of the female after about the fourth or fifth week. This difference together with the absence of the nipple and epithelial hood constitute the only characteristics that serve to distinguish the glands of the male from those of the female from the second to the beginning of the eighth week. As far as the structure of the remaining parts of the gland is concerned, one is unable to distinguish between the sexes. Figure 16 shows the milk-pore on the surface of the skin of a male rat of six weeks. The intra-epidermal part of the primary duct contains a shallow lumen. A solid mass of epithelial cells separates this lumen from that of the primary duct proper.

The connective-tissue sheath or mantle layer surrounding the milk-ducts becomes somewhat thicker as age advances. This sheath is quite thick around the primary and secondary ducts, but when traced toward the free end of a system of ducts gradually becomes thinner until it forms only an extremely thin layer around the terminal ducts. Previous to the third week, no elastic tissue fibers were observed in the mantle layer. At the end of the third week, however, Weigert's resorcin-fuchsin stain reveals a few elastic tissue fibers sprinkled among the other fibers of the mantle layer of the primary and secondary ducts. During the fifth, sixth, and seventh weeks more elastic fibers are present in the sheath of the primary and secondary ducts, and they are found in the sheath nearer the free end of a system of ducts than at three weeks. The true stroma is formed of loose connective-tissue in which various kinds of leucocytes are found. In no instance has an abnormal number of leucocytes been observed. The stroma also presents a great deal of adipose tissue. The connective tissue which occupies the nipple and epithelial hood is much more compact than in other parts of the gland.

About the third week the free ends of the milk-ducts of the abdominal and inguinal glands are observed to extend some distance into the inguinal fat pad where they send off numerous ramifications. As age advances the fat pad becomes more voluminous. It is not only thicker and longer, but wider than in the preceding stages. Hence its medial margin extends to the level of the abdominal and inguinal nipples. A great deal of adipose tissue has developed also in the stroma of the thoracic glands, so that the ramifications of many of the ducts are surrounded by fat cells.

Eight, nine, and ten weeks

It may be recalled that between the eighth and tenth week it was found in an earlier work (Myers, '16) that the ducts of the mammary gland of the female grow and proliferate very rapidly. Cleared preparations show that a tremendous number of new ducts bud out from the already existing ducts. These ducts bud off not only at the ends of the terminal ducts, but also as collateral ducts from the sides of all other ducts represented, including that part of the primary duct which lies parallel with the surface of the skin. In some of the ten weeks' stages the proliferation of ducts is not as marked as in those of nine weeks.

The nipple has enlarged somewhat and now at ten weeks more closely approaches the form of the nipple of the adult female rat. The groove at the base of the nipple has deepened apparently by the process of desquamation. In some individuals the tissue at the base of the nipple caudal to the first thoracic, cephalic to the second inguinal and lateral to the remaining nipples which in earlier stages connected the base of the nipple with the adjacent skin without the interruption of a furrow has now broken down so as to form a groove. This groove therefore extends completely around the base of the nipple. In fresh preparations the basal half of the nipple rests in the pocket formed by the groove. Such changes have not yet occurred, however, in all rats of nine and ten weeks of age (fig. 17). This furrow lies immediately superficial to the projection of the epithelial hood into which its cavity slightly extends. From the bottom of the furrow in some specimens may be seen the degenerating strands which were described in animals of two weeks (fig. 5). This furrow may contain debris which reacts to stains and appears under high magnification similar to the contents of the lumen of the intra-epidermal part of the primary duct.

The intra-epidermal part of the primary duct is still engorged with debris. Its walls are lined with stratified squamous epithelium of approximately the same thickness as that covering the surface of the nipple. At the end of the intra-epidermal part of the duct there is a gradual transition from the stratified squamous epithelium of this part of the duct to the epithelium of the primary duct proper. The walls of this part of the primary duct are composed of two or three layers of cells. The innermost layer is composed of low columnar cells, while the remaining layers are composed of cells of more irregular form. As the primary duct leaves the nipple and enters the deeper layers of the skin, its epithelium is arranged in two layers. The inner layer is composed of low columnar or high cuboidal cells containing elongated nuclei with their axes perpendicular to the lumen of the duct. The cells of the outer layer are somewhat irregular in size, shape, and position (fig. 9). In a longitudinal section of the primary duct, as it courses parallel with the surface of the skin, numerous processes may be seen some of which have lumina opening into the duct (fig. 6). These processes represent the collateral ducts. Some possess lumina and are lined with the same kind of epithelium as the primary duct, others are merely in the bud stage of formation and consequently have no lumina. Longitudinal sections of other ducts, as the secondary, tertiary, etc., likewise show numerous collateral ducts in various stages of formation. As the collateral ducts bud from the main ducts they carry with them a thin sheath of the connective tissue surrounding the main duct. Mitotic figures are numerous in the epithelial cells in these stages.

At this stage very little more than the ducts or excretory part of the gland is developed. However, at the end of some of the collateral ducts which branch from the secondary ducts one may observe several outpouchings which somewhat resemble a small cluster of grapes. Microscopic sections show that such outpouchings possess lumina which are surrounded by a layer of cuboidal cells, external to which may be seen an occasional somewhat flattened nucleus (fig. 10). These outpouchings may contain a slight amount of secretion, but they can hardly be called alveoli in the true sense of the word. True lobules are not yet present, although the above-mentioned clusters are doubtless an early indication of lobulation. The ramifications of ducts have become so numerous that they no longer occupy a single plane (fig. 19) as in the earlier stage (fig. 18), but form a rather dense arborization which in places reaches a thickness of 0.8 mm.

No true milk sinus, such as has been observed by various authors in several animal forms, has appeared in any of the described stages of the rat.

Elastic fibers are prominent in the mantle layer of the primary and secondary ducts. They are present in this layer of all the larger ducts, but tend to decrease in number as one passes toward the free end of a system of ducts. No elastic fibers have been observed in the sheath of the terminal ducts. The connective-tissue stroma is similar to that described in earlier stages except that it contains a greater amount of adipose tissue (fig. 19). The inguinal fat pad is so extensive that a thin layer of it now extends somewhat medial to the abdominal and inguinal nipples. Just under the nipple and partially occupying the epithelial hood is a condensed mass of fibrous tissue which becomes continuous with the less dense fibrous tissue of the nipple. Deep to and surrounding this mass of tissue is the loose stroma of the gland.

During this stage the blood-vessels in the stroma become enlarged so that in fresh specimens one notices a considerable increase in the vascularity of the gland as compared with the preceding stages.

As in the six weeks' stage, the primary duct in the male does not communicate with the exterior on account of a short solid cord of cells existing between its lumen and the very shallow lumen of the intra-epidermal duct. Moreover, it has been pointed out Myers ('17 b) that the marked growth of the female gland at the time of puberty does not usually occur in the male. The terminal enlargements appear similar to those in females of six and seven weeks. The walls of the ducts are composed of two layers of cells with the same arrangement as in the female. However, observations have revealed no indication of lobulation or developing alveoli in the male.

DISCUSSION AND CONCLUSIONS

In the following discussion, the nipple, the epithelial hood, milk-ducts, secretion in the new-born, and gland stroma will be successively considered.

The nipple pocket and epithelial hood

It will be recalled that in female fetuses of twenty days and six hours the nipple appears as a small elevation lying at the bottom of the mammary pit (Myers, '17). After the twenty days and six hours' stage the nipple grows so that at the time of birth in the female albino rat it nearly fills the mammary pit and appears as a somewhat round light area lying at the same level or very slightly elevated above the surrounding epidermis. At the end of the first week of postnatal life (fig. 12) the nipple is only a little more elevated than in the new-born. During the second week and especially in the last half of this week the nipple takes on a rapid development (fig. 13). At this time the nipple has reached a height of approximately 0.4 mm. From the third to the eighth week the nipple increases in size and gradually becomes somewhat conical in shape (fig. 15). During the eighth and ninth weeks, the time when the first ovulation normally occurs, the nipple begins to appear in size and form similar to the nipple of the adult virgin rat. In some individuals it now closely approaches the shape of a cone with a height of 1 mm., while in others it has not taken on its definitive conical form (fig. 17).

It is perhaps due to the fact that the nipple has no function to perform in the early life of the individual that it remains in a somewhat rudimentary state of development during this period. The rapid elevation during the second and third week may be closely related with the secretion that has already been produced in the milk-ducts. After the second and third weeks there seems to be nothing to stimulate a rapid growth of the nipple as it grows very gradually until near the time of puberty. As the first opportunity for pregnancy or the first ovulation approaches, the nipple takes on the shape and size of that of the adult virgin rat. This development of the nipple is apparently closely correlated with contemporaneous changes in the ovary as macroscopic observations show that Graafian follicles are rapidly growing in the early part of this stage. Furthermore, the vascular supply to the ovary very materially increases as the period of puberty is approached.

Rein ('82) found in the mammary glands of the rabbit two months after birth that the nipple presents a slight elevation in the form of the head of a pin. This elevation is about 1 mm. in height with a basal diameter of 1 to 1.5 mm. In rabbits of six months Schil ('12) found the nipples are conical elevations 1 to 1.5 mm. in height and 4 mm. in diameter at the base. Schil also states that the nipple is rarely indicated in the new-born human. Herz ('00), however, found that the nipple may or may not be elevated in new-born children.

While measurements on the nipples are lacking for many forms of animals, the present work together with general statements found in the literature are sufficient to substantiate the conclusion that the nipple remains in a rudimentary state during the early life of the individual. In the female it takes on its adult virgin form during the prepubertal and pubertal stages. In most animal forms a rudimentary nipple is present in the male at birth; however, it usually remains rudimentary throughout life. A more complete discussion on this subject may be found in an earlier paper (Myers, '17 b).

Epithelial hood

Immediately peripheral to the basal part of the developing nipple of the new-born the basal layer of the epithelium dips deeply to form the epithelial hood. Beginning about the second week of postnatal life, degenerative changes appear in the central part of the attached end of the epithelial hood. Such changes apparently have for their purpose the deepening of the groove which at first partially and later completely surrounds the base of the nipple.

At the time of birth a very shallow furrow may be seen surrounding the base of the nipple (fig. 1). A few days after birth this furrow in most cases has nearly disappeared. However, on the fifth day an apparently new depression (fig. 12) appears around only a part of the base of the nipple. As age advances, this depression or groove becomes deeper and extends around a greater part of the base of each nipple, until finally about the tenth week in some specimens it completely surrounds the base, thus forming a definite pocket in which approximately the basal half of the nipple rests.

Hair follicles appear lateral to the furrow surrounding the nipple (figs. 2 and 3), but have not been observed within this furrow.

Through the ten-weeks stage there is no indication of furrow or epithelial hood in the male albino rat.

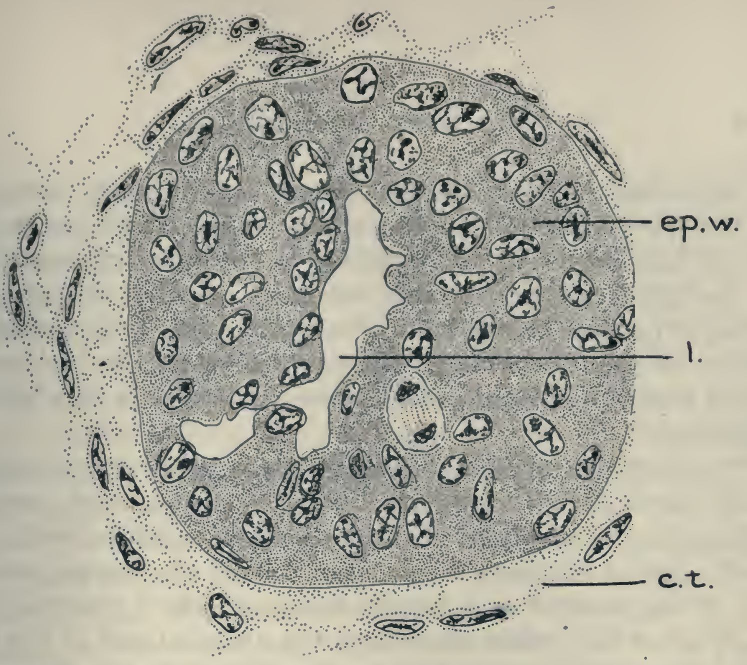
In 1876, Gegenbaur called attention to the fact that in Mus decumanus and Mus musculus the basal part of the nipples lies in a pocket-like depression of the integument. Gegenbaur believed the depression or pocket to be like the nipple pocket of marsupials. Rein ('82) studied the gland in a few of its stages in the white mouse and white rat. He concluded that the pocket surrounding the nipple in the adult has nothing to do with the nipple pocket of marsupials, but that it is identical with the marsupial pouch. Bresslau ('02, '10, and '12), in a series of studies, finally concluded that the sheath or pocket surrounding the nipple of the adult rodent and insectivorous mammals develops quite independently of the nipple pocket of marsupials or the marsupial pouch. The groove or sulcus at the base of the nipple in the albino rat was mentioned in my earlier work (Myers, '16), but its formation was not discussed at that time. The shallow groove which appears around the nipple in the new-born (fig. 1) is undoubtedly a remnant of the prenatal mammary pit which the growing nipple has not yet completely occupied. Sections through nipples of twelve hours, one day, two and three days after birth show that the sulcus around the new-born nipple has disappeared; in other words, the nipple completely fills the mammary pit. The very slight depression that surrounds only a portion of the base of the nipple at five days and continues to deepen and become more extensive until it forms a pocket containing nearly the basal half of the nipple at the tenth week apparently develops independently. We must therefore conclude that the pocket in which the base of the nipple of the adult virgin mouse and rat rests is not, as Gegenbaur believed, homologous with the nipple pocket of marsupials, but, as Bresslau suggested, it is an entirely independent structure which in the albino rat at least develops after birth.

The epithelial projection or hood was described in Mus decumanus and Mus musculus by Gegenbaur ('76). Later Rein ('82) observed such projections in white mice and white rats and described them from sections of fetuses as two solid epidermal thickenings which surround the mammary anlage. He described them as being somewhat curved with concavities directed toward each other. Rein found similar structures present in the mole. He believed that they have to do with the formation of the pocket around the nipple. Klaatsch ('84) also called attention to such projections. Bresslau ('12) observed these projections developing in Talpa europaea, white mouse, Mus musculus, Mus rattus, and Mus decumanus, and concluded that they develop into the secondary sheath or pocket of the nipple. In the albino rat wax reconstructions have been used by me to show that the epithelial projections seen in sections form a continuous hood around the primary duct. The attached end of this hood lies immediately peripheral to the base of the nipple. In view of the fact that the present work shows that the sulcus is deepened through degeneration and desquamation of the attached end of this hood, we must conclude that a portion of the sulcus is formed at the expense of the hood. In other words, the lateral wall of the attached part of the hood becomes the lateral wall of the deep part of the sulcus, while the medial wall of the attached end of the hood becomes the medial wall of the deep part of the sulcus. At present I am unable to say whether the sulcus forming the pocket around the nipple continues to deepen in the same manner during pregnancy and lactation until the free end of the hood is reached.

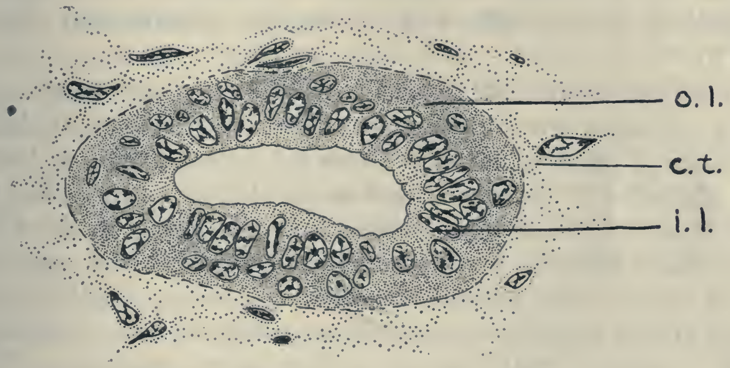
Milk-ducts

It will be recalled that the milk-ducts present several branches in female fetuses of about twenty days (Myers, '17 a). A considerable growth and branching occurs between this stage and the time of birth (compare fig. 12, p. 223, vol. 22, Am. Jour. Anat., with figs. 3 to 6, inclusive, pp. 361–365, vol. 19, Am. Jour. Anat.).

At the time of birth the lumina of the milk-ducts are not completely formed. The attached end of the primary duct for a short distance has no trace of a lumen. However, as one passes toward the deeper part of the duct one observes isolated lacunae. A little further along the duct one meets larger spaces which result from the flowing together of several lacunae (fig. 7). Finally, a slit-like lumen appears in the still deeper part of the duct, which extends through the secondary ducts. In the remainder of the ducts a fairly well-developed lumen is present. As age advances the lumen of the primary duct becomes com-



7



8

Fig. 7 Drawn from a cross-section through the primary milk-duct of the left first inguinal gland of a female albino rat at birth. Zenker's fixation; Weigert's iron-hematoxylin stain. Drawn with the aid of a camera lucida. *c.t.*, developing connective tissue forming sheath around the duct; *ep.w.*, epithelial wall composed of epithelial cells without definite arrangement into layers; *l.*, developing lumen. × 800.

Fig. 8 Drawn through a cross-section of a terminal duct (about 20μ from its termination) of a female albino rat of eleven days. Zenker's fixation; Weigert's iron-hematoxylin stain. Drawn with the aid of a camera lucida. *c.t.*, connective tissue; *i.l.*, inner or glandular layer of epithelial cells; *o.l.*, outer or myoepithelial layer of cells. × 800.

pletely formed. Near the highest part of the nipple elevation in the new-born female appears a shallow pit, the first indication of the milk-pore and the beginning of the intra-epidermal part of the primary duct. As age advances, this pit deepens and enlarges until at five or six weeks it comes in contact with the lumen of the primary duct proper. Thereafter the system of ducts is in direct communication with the surface through the milk-pore.

The walls of the ducts, especially the primary and secondary ducts, are quite thick at birth. The free half of each terminal enlargement is composed of a solid mass of epithelial cells. At birth some of the ducts toward the free end of a system present a wall composed of two definite layers of cells. After the first week all of the milk-ducts except the attached end of the primary duct are surrounded by an inner layer of very compactly placed low columnar or cuboidal cells and an outer layer of irregularly shaped cells with no regular arrangement (figs. 8 and 9).

Ancel and Bouin ('09) found in rabbits as many as ten milk-ducts radiating from the nipple. Each duct in animals before the stage of puberty, however, does not exceed 2 mm. in length. The diameter of the gland reaches about 3 or 4 mm. The fact that there is a single duct leading from the nipple in the albino rat perhaps accounts for the much greater length and branching of the ducts in the prepuberty stage. Steinhaus ('93) found in young guinea-pigs that the glands are composed only of excretory ducts. Dulcert ('93) arrived at the same conclusion. They did not observe many mitotic figures. In a heifer of one year Dulcert ('93) reported only excretory canals and observed no mitotic figures. O'Donoghue ('12) found in the deeper parts of the glands of Dasyurus a few structures of doubtful significance which may or may not represent mitotic figures, but he found that the actual formative growth of the gland does not appear to start until after ovulation.

Langer ('51), Raubitschek ('04), and others have reported true alveoli in the mammary gland of human after birth and before puberty. Berka ('12), however, was unable to find true alveoli present in these stages. Schil ('12) states that from birth until shortly before the puberty period the mammary gland of the rabbit undergoes very little or no growth, that the slight elongation of the ducts is due to a stretching produced by the developing stroma. He states that there are two facts which characterize this period: first, the gland maintains the development already reached at birth; second, some of the cells produce a very slight secretion. These facts, he believes, prove that the gland does not receive any impulse of growth or secretion. However, Schil thinks that during this entire period the glands are under the influence of the ovaries, for after double ovariotomies in young animals the free parts of the ducts not only fail to produce a slight secretion, but disappear so that the gland is reduced to some short ducts centering around the nipple. On the other hand, he cites the experiments of Knauer and Foges which show that after simple hysterectomies without ablation of the ovaries the gland remains as in normal or control animals. Schil concludes, therefore, that the presence of the ovary is necessary to maintain the mammary gland in the stage it has reached at the time of birth.

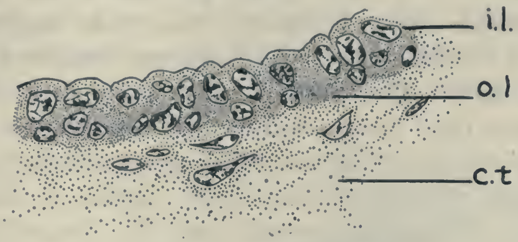
My previous work (Myers, '16) shows beyond doubt that the ducts do grow and proliferate a great deal after birth and before the period of puberty is reached. According to the castration experiments of Steinach ('12) and others, it seems probable that this gradual growth in the milk-ducts of the albino rat from birth to a short time before puberty is under the influence of the ovaries. Further castration experiments are being attempted to confirm this point. It has been pointed out, however, (Myers, '17 b) that the milk ducts of the male albino rat undergo a similar but less extensive growth and proliferation from the time of birth to puberty. If, as Schil believes, the presence of the ovaries is necessary even to maintain the gland of the rabbit in its infantile state until puberty, we are at present unable to locate the factor which stimulates the growth of the glands of the male during this period.

The rapid growth and proliferation of the milk-ducts at the time of puberty has been mentioned by a number of investigators, but only a few have made a detailed study of this growth. Langer ('51) called attention to the growth of ducts at this period in human. Ancel and Bouin ('10 and '11) showed that during the period of puberty the mammary gland in the rabbit reaches a diameter of 2 to 3 cm. Frank and Unger ('11) showed that a very decided growth occurs in the mammary glands of rabbits during puberty. Schil ('12), in a careful series of observations on rabbits, found that the glands gradually increase throughout the first period of heat, after which they undergo slight regressive changes until the next period of heat. If pregnancy does not occur, the glands proliferate with each heat. The regression following heat depends upon the interval between two periods. Brouha ('05) has shown in Vespertilio murinus, in which there appears a single ripe follicle each year, that between two pregnancy periods the gland may return to the infantile stage. The fact that the glands of some rats at ten weeks of age show less development than those in others at nine weeks may be due to slight regressive changes since the first ovulation, as this ovulation may occur earlier than nine or ten weeks (Lantz, '10; Jackson, '12). However, owing to the fact that careful studies on the oestrus cycle in the albino rat have not yet been published, the question as to changes in the mammary gland between and during successive periods of heat has been omitted in this study.

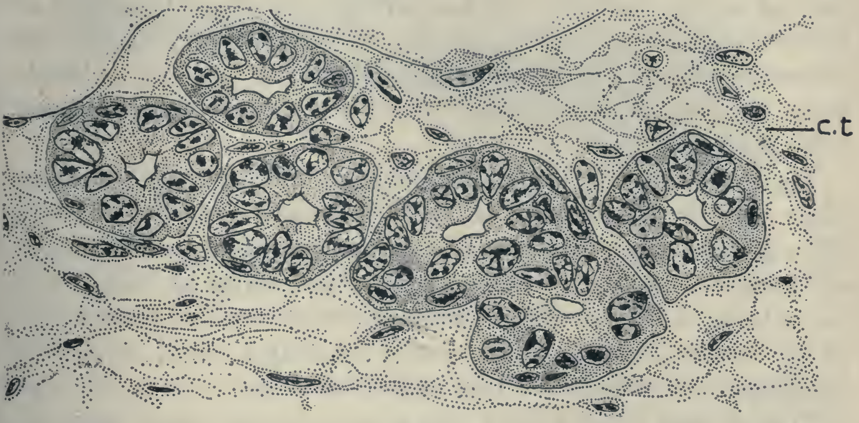
The present study, my previous work (Myers, '16), and the findings of the above-mentioned authors lead to the conclusion that there is in the female of several animal forms a very marked development of the mammary gland at the time of puberty. Since such development does not ordinarily occur in the glands of the male or in castrated females, it seems probable that these changes are at least stimulated by simultaneous changes in the ovaries.

The time and manner of formation of the lumina of the milk-ducts were previously discussed (Myers, '17 a). Suffice it to say here that the method of formation of the lumen already begun in the fetus is continued in the postnatal stages until the lumina are completely developed. The lumen of the intraepidermal part of the primary duct forms an exception, however, as it develops by the process of degeneration and desquamation.

The lining of the milk-ducts in the adult virgin human has been described by von Ebner as consisting of a single layer of epithelial cells. Starling and Lane-Claypon ('06) also reported a single layer of epithelium surrounding the ducts in virgin rabbits. Rein ('82) divided the duct in rabbits of two months into



9



10

Fig. 9 Drawn from a cross-section of a secondary milk-duct of a female albino rat of nine weeks. Zenker's fixation; Weigert's iron-hematoxylin stain. Drawn with the aid of a camera lucida. *c.t.*, connective tissue; *i.l.*, inner or glandular layer of epithelium; *o.l.*, outer or myo-epithelial layer. × 800.

Fig. 10 Drawn from a section through the terminal branches of a collateral duct of a female albino rat of nine weeks. Zinker's fixation; Weigert's iron-hematoxylin stain. Drawn with the aid of a camera lucida. *c.t.*, connective tissue. The cross-sections through the six terminal parts show in general a single layer of epithelium. An occasional flattened nucleus represents the outer or myo-epithelial layer. × 800.

three parts: first, the short excretory duct which extends from the milk-pore to a noticeable enlargement; second, the enlargement or milk-sinus, and, third, the milk-duct or canal. He found the excretory duct lined with a stratified pavement epithelium, while the sinus and milk-ducts possess two epithelial layers. Benda ('94) found the epithelium near the attached parts composed of two layers of cells, while in the deeper and free parts of the ducts the epithelium was described as consisting of a single layer. Raubitschek ('04) found in a human female of seven days that the epithelium and the ducts is composed of two layers. Brouha ('05) described this epithelium as consisting of two layers throughout the entire system of ducts in virgin Vespertilio murinus, the rabbit, and the domestic cat. Schil ('12) observed two distinct layers of cells around the ducts before the stage of puberty. O'Donoghue ('12) confirms Brouha's finding in the domestic cat and writes concerning Dasyurus as follows: "As in the foetus, so in the adult Dasyurus, all the branching tubules of the mammary gland are lined by a double layer of cells, at any rate in the resting animal, and for some time after ovulation."

The fact that a few ducts were observed in the early postnatal stages of the albino rat with small areas covered with a single layer of epithelium probably has no significance as in later stages all ducts observed were lined with a double layer of cells. The outer layer of cells doubtless represents the so-called basket cells or myo-epithelial cells of the active mammary gland, while the inner layer represents the true glandular cells.

True alveoli lined with a single early of cells have been reported in the glands of various animal species before puberty by several writers. Such alveoli or acini have probably been observed, as there is no reason to doubt that through individual variation a few acini might develop just as a mature Graafian follicle sometimes appears in the ovary of the human fetus or new-born. However, Schil ('12) concluded that during this period the mammary gland in the rabbit ordinarily presents no glandular acini. O'Donoghue ('12), after studying the glands of Dasyurus, made the following statement: "The true secretory alveoli of the gland with their simple epithelial lining do not make their appearance until the last few days of pregnancy or until some time after ovulation when this has not been followed by fertilization." The work of these investigators, the present work, and my previous work (Myers, '16) prove that in certain animals true alveoli do not usually appear before puberty.

It has been proved quite conclusively that true secretory alveoli do appear after the first ovulation. In fact, Ancel and Bouin ('11) and Schil ('12) showed that in the rabbit the mammary gland develops after each ovulation not followed by pregnancy to the same extent as it develops during the first half of pregnancy. O'Donoghue ('12) and Hartman ('18) reported somewhat similar changes in the mammary glands of Dasyurus and the opossum. Most of the investigators believe this development is due to the corpus luteum. The enlarged terminations of the ducts which resemble alveoli in some ten-week rats were perhaps developed during the first period of heat or some time after the first ovulation.

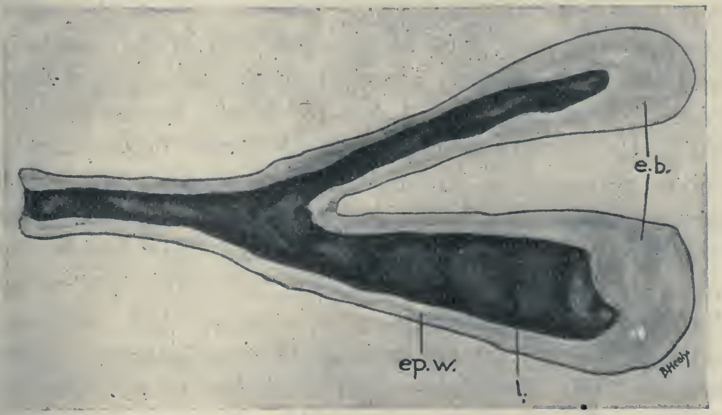


Fig. 11 Internal view of a wax model reconstructed from two terminal duct and end-buds of the right first thoracic gland of a female albino rat of seven weeks. *e.b.*, end-bud; *ep.w.*, epithelial wall; *l.*, lumen. × 100.

Secretion in the new-born

It will be recalled that twelve hours after birth a slight secretion appears in the lumina of the milk-ducts. This secretion is more abundant at the fourth and fifth days and remains present in approximately the same amount during the first week. At no time does it completely fill the lumina of the ducts. After the second week the secretion gradually appears present in smaller amounts; however, at the ninth and tenth weeks there is still some secretion to be found in the lumina. There is only a very occasional free epithelial cell present in the secretion. During the first few days of postnatal life such cells manifest no signs of degeneration, however, later a few were observed undergoing degeneration. A few red blood corpuscles were observed in the lumina of the ducts at twelve hours after birth, but none appeared in any of the later stages. An occasional leucocyte may be seen in the lumina at any stage from birth to ten weeks. In none of the stages studied could fat be demonstrated in the lumina or the epithelial cells lining the ducts.

The secretion in the new-born or witches' milk has been observed in human by a large number of investigators. According to Brouha ('05), Aristotle called attention to it. The writers of the second half of the nineteenth and the early part of the twentieth centuries presented different theories concerning the origin of this secretion. For example, De Sinety ('75), Rein ('82), Barfurth ('82), Czerny ('90), Unger ('98), Schalachta ('04), Brouha ('05), Berka ('11), Schil ('12), and others regarded it in the human new-born as a true milk secretion, while Kölliker ('54), Milne Edwards ('70), and Raubitschek ('04) believed it is the product of necrobiotic changes which result in the formation of the lumina. Still another view is that of Keiffer ('02), who described the formation of milk in the new-born as a pathological process.

Several theories have been offered to account for the stimulus which excites the secretion in the new-born human. The fact that Brouha concluded that the new-born secretion in well-developed human may completely precede the birth of the child and that Schil observed true milk secretion in the ducts of a human fetus at the beginning of the eighth month, proves fairly conclusively that a secretion capable of stimulating the glands to action is formed in the placenta or ovary of the mother and transmitted to the blood of both mother and offspring, thus bringing about the formation of milk in both maternal and fetal mammary glands.

Berka ('11) believes the contents of the lumina of the milk-ducts through the virgin stage is a part of the witches' milk which has been retained in the ducts. On the other hand, Schil ('12) thinks this results from a constant slight secretory activity of the epithelial cells. O'Donoghue ('12), after studying Dasyurus agrees with Schil in the following:

> A trace of a secretion somewhat resembling colostrum is always to be found as a coagulum in the lumen of the tubules and ducts until it is removed by the more active secretion of colostrum or milk. It would appear, then, that the gland, quite apart from the proper milk flow, is the seat of slow secretory activity, although this secretion is quite different in microscopic appearance from true milk.

Aristotle is said to have observed witches' milk in the goat. Creighton ('78) reported it in the guinea-pig in the following manner:

> The fluid expressed from the nipple of the new-born guinea-pig had the appearance of a watery kind of milk; on microscopic examination, the milk-globules of ordinary milk were not found, but a more uniform fluid mass irregularly broken up under the cover-slip into large or small drops, and without any mixture of cellular elements.

Barfurth noticed such a secretion in the guinea-pig, bitch, and rabbit and regarded it as a coagulum resulting from simple transudation. After studying it in Vespertilio murinus, rabbit, and the domestic cat, Brouha ('05) came to the following conclusions in regard to these forms; "la sécrétion natale existe également, mais elle n'aboutit pas, au moins dans les trois premières semaines de le vie, à une véritable lactation." Schil ('12) observed secretion in the ducts of rabbit embryos as well as postnatal stages. No secretion has been observed in the ducts of rat fetuses by me. Brouha's work and the present study lead to the conclusion that in the rabbit, cat, bat, and white rat the so-called new-born secretion does not contain fat and hence is not a true lactation as is found in human. This failure to produce true milk in some of the lower forms may be due to the feeble development of the mammary gland in the new-born. The lumina are not completely formed at birth in the rat, cat, and rabbit, therefore the cells may be unable to produce a true milk secretion when the stimulus is received. On the other hand, Brouha found the lumina quite well represented in Vespertilio murinus at the time of birth. The question then arises as to whether the stimulus which produces a true lactation in the human new-born is actually present in the lower forms of animals. Further experimental evidence is necessary to decide this question.

Gland stroma

Attention was called to the fact that the stroma in the fetus is divisible into the mantle layer closely surrounding the ducts and the true stroma between the ducts. This arrangement of the stroma persists through the ten weeks' stage. The mantle layer increases somewhat in thickness as age advances. This layer diminishes in thickness as one passes from the primary to the terminal ducts. About the third week of postnatal life a few elastic fibres appear in the mantle layer surrounding the primary and secondary ducts. In later stages elastic fibers are more numerous and extend further toward the free end of a system of ducts. The true stroma is at first composed of loose connective tissue, but soon after birth there appears such a tremendous invasion of fat that the mantle layer of the ducts is almost entirely surrounded by fat cells.

Free red corpuscles were observed in the stroma twelve hours after birth, but were not seen in any of the later stages. White blood-cells were observed in the true stroma in all stages studied, but in no stage did they appear in greater numbers than they are usually found in the neighboring tissues.

Berka ('11) called attention to the presence of elastic fibers in the mantle layer of the virgin human ducts. Schil ('12) observed elastic fibers in rabbits six months of age and at the age of puberty.

Red blood corpuscles were observed in the true stroma of the glands of premature and mature infants by Schlachta ('04). Brouha ('05) found capillary hemorrhage exists in the stroma of all infants that died from abrupt asphyxia. He noticed, however, that in infants dying from other causes there may be a considerable extravasation of red blood corpuscles. Both of these authors concluded that the red corpuscles probably reached the stroma through the process of diapedesis. Owing to the presence of red blood-cells in the mammary-gland stroma of the albino rat in comparatively small numbers, it is possible that they reach the stroma through the process of diapedesis. However, it must be borne in mind that during parturition and the removal of the skin there is considerable danger of trauma.

Several authors have reported the presence of an abnormally large number of leucocytes in the true stroma of the mammary gland at or soon after birth. Czerny ('90) believed they play an important rôle by entering the lumina of the ducts and carrying the secretion into the lymphatics. Schlachta ('04) was unable to confirm Czerny's finding. Brouha ('05), however, found the immigration of leucocytes in the gland stroma to be less marked during the activity of the new-born gland than before or after such activity. The fact that the secretion in the albino rat is very slight and never becomes a true milk secretion probably accounts for the absence of an infiltration of leucocytes.

SUMMARY

The results of the present study on the mammary gland of the male and female albino rat from birth to ten weeks of age may be summarized briefly as follows:

1. Owing to the thickening of the epidermis over the developing nipple of the new-born female albino rat, the mammary-gland area appears lighter than the surrounding epidermis. In the male no epidermal thickening is present, hence the mammary-gland area is not visible.

The nipple elevation is oblong in shape and very slightly elevated at the fourth or fifth day. At the end of the second week it is still oblong, but much more elevated. After the second week its growth is a gradual one until the time of puberty, when it takes on the size and shape of the adult virgin nipple.

2. The sulcus around the nipple in the new-born female rat is apparently a remnant of the prenatal mammary pit. During the early days of postnatal life this sulcus disappears, but on the fourth or fifth day an apparently new depression makes its appearance. This depression deepens so that by the tenth week there may be developed a definite pocket in which the basal part of the nipple is located.

3. The epithelial ingrowth (hood) at birth presents a smooth and quite regular outline. Two or three days after birth, however, the stratum germinativum becomes somewhat thickened in some places, thus forming short processes. Such processes appear on both the outer and inner surfaces of the hood throughout the stages studied. The central portion of the proximal part of the hood apparently degenerates, thus deepening the sulcus around the base of the nipple.

4. Hair follicles are numerous in the integument peripheral to the hood and sulcus, but have not been observed nearer the nipple.

5. Near the highest part of the nipple there appears, a few days after birth, a slight excavation—the developing milk-pore, communicating with the intra-epidermal part of the primary duct. At two weeks there is a slight connection between this cavity and the lumen of the primary duct. About the sixth week a complete connection is established with the primary duct. When the nipple becomes cone-shaped, the milk-pore appears near the apex.

6. The lumina of the milk ducts are apparently formed by the process of rearrangement of the cells. The lumen of the primary duct is not completely formed until after the second week. The other ducts possess fairly well-developed lumina at the time of birth.

7. The walls of the milk-ducts are for the most part lined with a two-layered epithelium. The inner or glandular layer being composed of cuboidal to low columnar cells, while the outer (or so-called myo-epithelial layer) is composed of cells irregular in size, shape, and arrangement. At nine or ten weeks some of the terminal processes show indications of developing into alveoli. The walls of the intra-epidermal part of the primary duct are lined with stratified epithelium similar to that covering the surface of the nipple.

8. Masses of subcutaneous fat develop soon after birth. Such masses increase in size as age advances, and it is in this fat that many of the milk-ducts ramify.

9. The stroma is divisible into its usual parts. The mantle layer forms a thin sheath immediately surrounding the milk-ducts. At the third week and thereafter elastic tissue fibers were visible in this layer surrounding the larger ducts. The true stroma is formed of a loose connective-tissue network in the early stages, but later shows a very marked infiltration of fat. Extending through the adipose tissue may be seen an occasional fair-sized connective-tissue lamina.

10. In no stage was an infiltration of leucocytes observed in the stroma.

11. A slight secretion appears in the lumina of the milk-ducts soon after birth. In no case has such secretion appeared in sufficient quantities to cause distention of the ducts. A trace of secretion may be seen in the milk-ducts through the ten weeks' stage. Specific fat stains fail to reveal fat in the secretion of the milk-ducts or in the epithelial walls, notwithstanding the fact that much fat was seen in the true stroma outside the walls of the ducts.

BIBLIOGRAPHY

Ancel, P., et Bouin, P. 1909 Sur la fonction du corps jaune. Action du corps jaune vrai sur la glande mammaire (troisième note préliminaire). Compt. rend. Soc. Biol., T. 66, p. 605. 1911 Recherches sur les fonctions du corps jaune gestatif. 11. Sur le déterminisme du développement de la glande mammaire au cours de la gestation. Journ. de phys. et de path. générale, no. 1, p. 31.

Barfurth, Dietrich 1882 Zur Entwickelung der Milchdrüse. Inaug. Diss., Bonn.

Bell, E. T. 1914 On the differential staining of fats. Journal of Pathology and Bacteriology, vol. 19, p. 105.

Benda, L. 1894 Das Verhältnis der Milchdrüsen zu den Hautdrüsen. Dermatolog. Zeitschr., Bd. 1.

Berka, F. 1911 Die Brustdrsüe verschiedener Altersstufen und während der Schwangerschaft. Frankfurter Zeitschrift für Pathologie, vol. 8.

Bresslau, E. 1902 Beiträge zur Entwickelungsgeschichte der Mammarorgane bei den Beutelthieren. Zeitschrift f. Morphologie u. Anthropologie, Bd. 4.

1910 Der Mammarapparat (Entwicklung und Stammesgeschichte). Ergebn. d. Anat. u. Entw., Bd. 19.

1912 Die Entwickelung des Mammarapparates der Monotremen, Marsupialier und einiger Placentalier. III. Entwickelung des Mammarapparates der Marsupialier, Insectivoren, Nagethiere, Carnivoren und Wiederkäuer. Semon's Zoolog. Forschungsreisen, Bd. 5.

Brouha, Dr. 1905 Recherches sur les diverses phases du développement et de l'activite de la mamelle. Archives de Biologie, T. 21.

Creighton, Charles 1878 Physiology and Pathology of the breast. London.

Czerny, A. 1890 Über die Brustdrüsensecretion beim Neugeborenen u. über das Verhältniss der sogenannten Colostrumkörperchen zur Milchsecretion. Festschr. d'Henoch.

De Sinety, 1875 Recherches sur la mamelle des enfants nouveau-nés. Arch. de physiol. norm. et patholog., T. 2.

Dulcert 1893 Étude histologique de la sécrétion lactée. Thèse de Montpellier.

Foges, Arthur 1905 Zur physiologischen Beziehungen von Mamma und Genitalien. Zentralblatt f. Physiol., Bd. 19.

1908 Zur Physiologischen Beziehungen von Mamma und Genitale. Wien. klin. Woch., Bd. 21.

Frank, R. T., and Unger, A. 1911 An experimental study of the causes which produce the growth of the mammary gland. Archives of internal medicine, vol. 7.

Gegenbaur, C. 1876 Zur genaueren Kenntniss der Zitzen der Säugethiere. Morpholog. Jahrb., Bd. 1.

Hartman, Carl 1918 Personal communication. Work in progress at The Wister Institute of Anatomy, Philadelphia.

Herz, P. 1900 Klinische Untersuchungen an 100 Neugeborenen. Inaug. Diss. Freiburg i. B., S. 1–61.

Jackson, C. M. 1912 On the recognition of sex through external characters in the young rat. Biol. Bull., vol. 23.

Keiffer, H. 1902 La glande mammaire chez le foetus et chez le nourrisson. Bull. Soc. Belge de Gyn. et d'Obstet., T. 13.

Klaatsch, H. 1884 Zur Morphologie der Säugethierzitzen. Morph. Jahrb., Bd. 9.

Knauer, Emil 1900 Die Ovarientransplantation. Arch. f. Gyn., Bd. 60.

Kölliker, Th. 1850 Mikroskopische Anatomie, Bd. 2.

LANE-CLAYPON, MISS J. E., AND STARLING, E. H. 1906 An experimental enquiry into the factors which determine the growth and activity of the mammary glands. Proc. of the Royal Society, Series B, vol. 77.

LANGER, CARL 1851 Über Bau und Entwicklung der Milchdrüse. Denkschrift der Wiener Akad. d. Wiss., Bd. 3.

LANTZ, DAVID E. 1910 The natural history of the rat. In "The rat and its relation to the public health," by various authors. P.H. and M.H. Service, Washington, D. C.

LOEB, LEO, AND HESSELBERG, CORA 1917 The cyclic changes in the mammary gland under normal and pathological conditions. Jour. of Experimental Medicine, vol. 25.

MILNE-EDWARDS 1870 Leçons sur la physiologie et l'anatomie comparée de l'homme et des animaux. Paris, T. 9.

MYERS, J. A. 1916 Studies on the mammary gland. I. The growth and distribution of the milk-ducts and the development of the nipple in the albino rat from birth to ten weeks of age. Am. Jour. Anat., vol. 19. (Abstract also published in the Anat. Rec., 1916, vol. 10, p. 230.)

1917 a Studies on the mammary gland. II. The fetal development of the mammary gland in the female albino rat. Am. Jour. Anat., vol. 22. (Abstract also published in the Anat. Rec., 1917, vol. 11, p. 390.)

1917 b Studies on the mammary gland. III. A comparison of the developing mammary glands in male and female albino rats from the late fetal stages to ten weeks of age. Anat. Rec., vol. 13. (Abstract also published in the Anat. Rec., 1917, vol. 11.)

1918 The histology of the mammary gland in the male and female albino rat from birth to ten weeks of age. (Abstract.) Anat. Rec., vol. 14, p. 46.

O'DONOGHUE, CHARLES H. 1912 The growth-changes in the mammary apparatus of Dasyurus and the relation of the corpora lutea thereto. Quarterly Journal of Microscopical Science, vol. 57.

RAUBITSCHEK, H. 1904 Über die Brustdrüsen menschlicher Neugeborenen. Zeitsch. f. Heilkund., Abth. f. pathol. Anatomie, Heft 1.

REIN, G. 1882 Untersuchungen über die embryonale Entwicklungsgeschichte der Milchdrüse. Archiv für mikr. Anatomie, Bd. 20 and 21.

SCHLACHTA 1904 Beiträge zur mikroskopischen Anatomie der Prostate u. Mamma des Neugeborenen. Arch. f. mikros. Anat. u. Entwick.

SCHIL, L. 1912 Recherches sur la glande mammaire, sur les phases qu'elle présente au cours de son évolution et leur determinisme. Thèse, Lyon.

STEINACH, E. 1912 Willkürliche Umwandlung von Säugetier-Männchen in Tiere mit ausgeprägt weiblichen Geschlechtscharakteren und weiblicher Psyche. Archiv für die gesammte Physiologie (Pflüger's).

STEINHAUS, JULIUS 1892 Die Morphologie der Milchabsonderung. Arch. f. Ant. u. Physiol., Suppl. Bd.

UNGER, E. 1898 Beiträge zur Anatomie u. Physiologie der Milchdrüse. Anat. Heft.

VON EBNER 1902 Handbuch der Geweblehren des Menschen von Kölliker, Bd. 3.

PLATE 1

EXPLANATION OF FIGURES

12 External view of wax model reconstructed from the right third thoracic gland of a female albino rat of five days. Reconstruction built by A. H. Nerad. *ep.*, epidermis; *n.*, nipple eminence; *s.*, sulcus. × 37½.

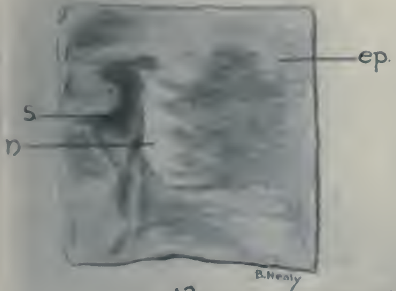
13 External view of a wax model reconstructed from the right second inguinal gland of a female albino rat of two weeks. Reconstruction built by A. F. Branton. *ep.*, epidermis; *m.po.*, milk-pore; *n.*, nipple; *s.*, sulcus. × 37½.

14 Internal view of a wax model reconstructed from the right second inguinal gland of a female albino rat of two weeks. Reconstruction built by A. F. Branton. *ep.*, epidermis; *ep.in.*, epithelial ingrowth; *p.d.*, primary duct. × 37½.

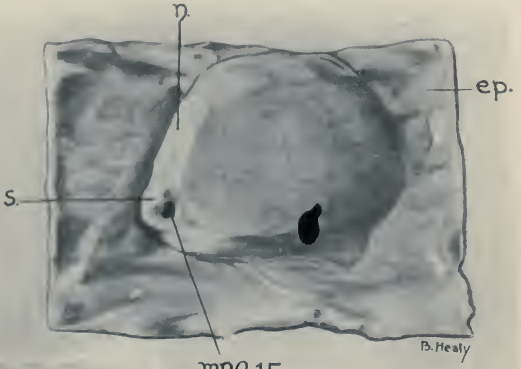
15 External view of a wax model reconstructed from the right second inguinal gland of a female albino rat of six weeks. Reconstruction built by Nellie Pederson and Frieda Radusch. *ep.*, epidermis; *m.po.*, milk-pore *n.*, nipple; *s.*, sulcus. × 37½.

16 External view of a wax model reconstructed from the right abdominal gland of a male albino rat of six weeks. *ep.*, epidermis; *m.po.*, milk-pore. × 37½.

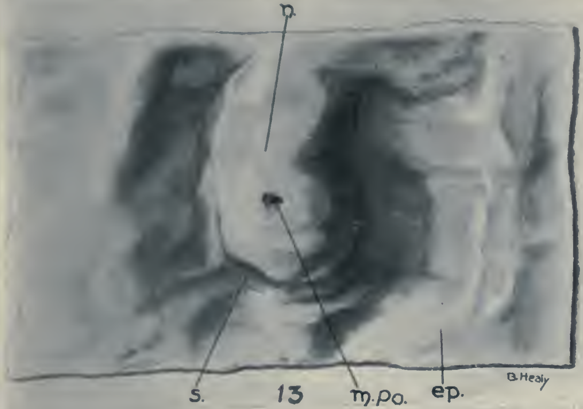
17 External view of a wax model reconstructed from the right first inguinal gland of a female albino rat of nine weeks. Reconstruction by M. H. Litman and A. F. Branton. *ep.*, epidermis; *m.po.*, milk-pore; *n.*, nipple; *s.*, sulcus. × 37½.



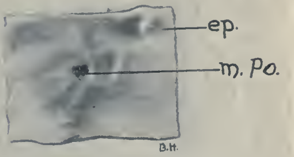
12



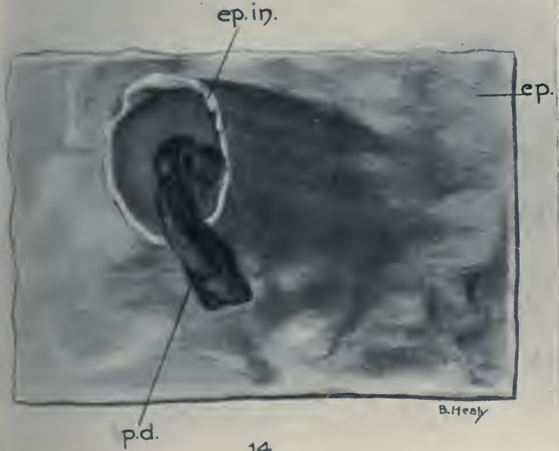
15



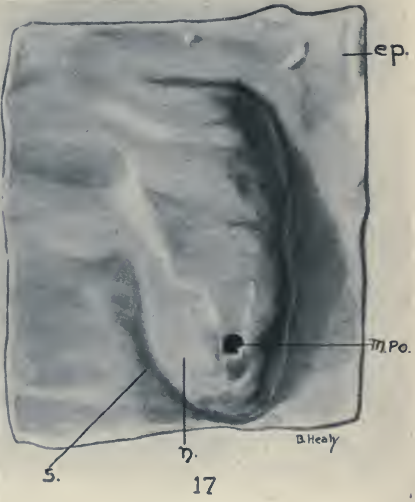
13



16



14



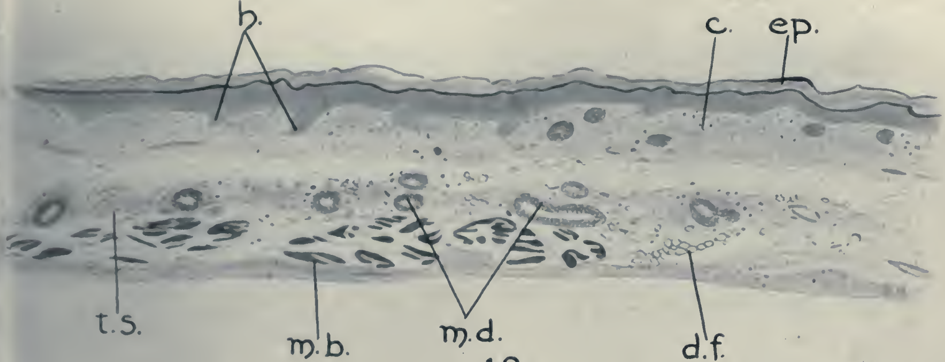
17

PLATE 2

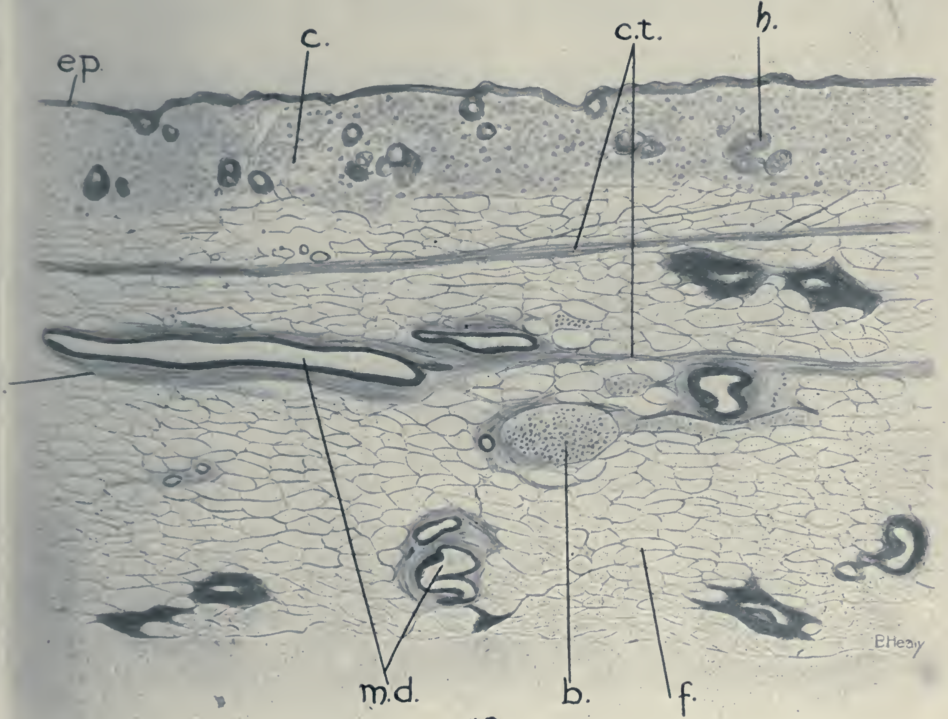
EXPLANATION OF FIGURES

18 Drawn from a section through the right first inguinal gland of a female albino rat at birth. Zenker's fixation; Mallory's connective-tissue stain. *c.*, corium; *d.f.*, developing fat cells; *ep.*, epidermis; *h.*, hair follicles; *m.b.*, muscle bundle; *m.d.*, milk-ducts; *t.s.*, tela subcutanea. × $83\frac{1}{3}$.

19 Drawn from a section through the right first inguinal gland of a female albino rat of nine weeks. Zenker's fixation; Mallory's connective-tissue stain. *b.*, blood vessel; *c.*, corium; *c.t.*, connective-tissue lamina; *ep.*, epidermis; *f.*, fat cells; *h.*, hair follicles; *m.l.*, mantle layer; *m.d.*, milk-ducts. × $83\frac{1}{3}$.



18



19

No. 8.

Studies on the Mammary Gland

V. THE EFFECTS OF INANITION ON THE DEVELOPING MAMMARY GLANDS IN MALE AND FEMALE ALBINO RATS FROM BIRTH TO TEN WEEKS OF AGE

J. A. MYERS, M.S., Ph.D.
MINNEAPOLIS

·3,

STUDIES ON THE MAMMARY GLAND

V. THE EFFECTS OF INANITION ON THE DEVELOPING MAMMARY GLANDS IN MALE AND FEMALE ALBINO RATS FROM BIRTH TO TEN WEEKS OF AGE *

J. A. MYERS, M.S., PH.D.
MINNEAPOLIS

In a previous work[1] attention was called to the fact that the postnatal development of the milk ducts probably depends to some extent on the body weight of the animal. The present work is an attempt to show: (1) to what extent the postnatal development of the milk ducts may be altered by changes from the normal body weight of the animal; (2) the effect of severe inanition on the development of all parts of the mammary gland; (3) the recovery of the mammary gland in animals on refeeding after inanition.

MATERIAL AND TECHNIC

The material used in this study was kindly presented to me by Dr. C. A. Stewart,[2] who made a study on the central nervous system of the same animals. The series of rats used included more than twenty males and females ranging in age from birth to 10 weeks (one or two individuals being somewhat older). The control rats were allowed to grow normally while the test animals were held at or near birth weight for various periods of time. This was done by removing the test rats from the mother at successive periods. To restrict the weight after weaning the rats were fed small amounts of whole wheat (Graham) bread, soaked in whole milk. After such periods of starving, a few females were refed until they reached the weight of the normal or control animal at the time of killing.

In most cases comparisons were made from test and control rats of the same litter. However, in some cases the glands in a control rat of the same litter as the test animal were not available; hence it became necessary to compare the glands of the test rats with those of apparently normal animals of other litters of the same age. In both test and control animals some of the glands were sectioned serially, the sections being 10 microns in thickness, and stained in Heidenhain's iron hematoxylin stain or Mallory's connective tissue stain.

So far as possible the glands were removed from the epidermis with the connective tissue layers of the skin and cleared according to the method used

---

* From the Institute of Anatomy, University of Minnesota.

[1] Myers, J. A.: Studies on the Mammary Gland. III. A Comparison of the Developing Mammary Glands in Male and Female Albino Rats from the Late Fetal Stages to Ten Weeks of Age, Anat. Rec. **13**: 1917 (Abst. in Anat. Rec. **11**: 1917).

[2] Stewart, C. A.: Weights of Various Parts of the Brain in Normal and Underfed Albino Rats at Different Ages, J. Comp. Neurol. **25**:511, 1918.

by Lane-Claypon and Starling.[3] In rats held at slightly above birth weight, however, it was extremely difficult to remove the connective tissue layer of the skin without tearing it or injuring the mammary glands.

OBSERVATIONS

1. *Effects of Inanition.—Newborn.*—In a normal new-born female albino rat of about 4.5 gm., the mammary glands (Fig. 1) show a considerable branching of the milk ducts. (Only two of the six glands on each side are shown in the figure. For complete description, see Myers.[4]) Quaternary ducts are present in all of the glands examined and in most individuals the fifth, sixth and even the seventh divisions of the ducts have occurred. Terminal end-buds are fairly prominent. Collateral ducts (short side branches) are present in small numbers and are chiefly located near the free end of the ducts of a gland. They are very rare on any of the main ducts nearer the nipple than

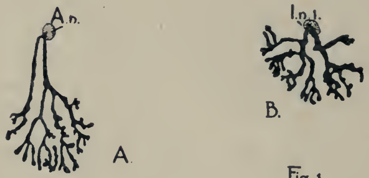


Fig. 1.

Fig. 1.—Drawn from a cleared preparation (internal view) of a new-born female albino rat (weight 4.5 gm.) to show distribution and relations of ducts of right abdominal gland (A), and right first inguinal gland (B) × 5. A.n., abdominal nipple; I.n.1, first inguinal nipple; p.d., primary duct; s.d., secondary duct; t.d., tertiary duct; tr.d., terminal duct; e.b., end-bud.

Fig. 2.

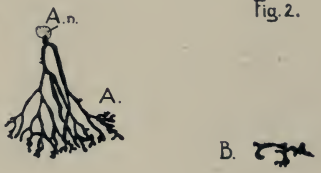


Fig. 2.—Same as Figure 1, but from animal that was starved so as to weigh only 3.6 gm. at eight days, × 5. The greater part of the first inguinal gland (B) was lost in making the cleared preparation.

---

[3] Lane-Claypon, Miss J. E., and Starling, E. H.: An Experimental Inquiry Into the Factors which Determine the Growth and Activity of the Mammary Glands, Proc. Roy. Soc. M. and S., Series B **77**: 1906.

[4] Myers, J. A.: Studies on the Mammary Gland. I. The Growth and Distribution of the Milk Ducts and the Development of the Nipple in the Albino Rat from Birth to Ten Weeks of Age, Am. J. Anat. **19**: 1916 (Abst. in Anat. Rec. **10**:20, 1916). Myers, J. A.: Studies on the Mammary Gland. II. The Fetal Development of the Mammary Gland in the Female Albino Rat, Am. J. Anat. **22**: 1917 (Abst. in Anat. Rec. **11**:390, 1917).

the quaternary ducts. The branching of the milk ducts in the new-born male is essentially the same as in the female.

It was previously shown[5] that the nipple of the new-born female albino rat is only slightly elevated above the surface of the surrounding epidermis, and that in some cases there is around the nipple a shallow sulcus, which was regarded as a remnant of the mammary pit of the fetus. The epidermis covering the nipple is considerably thickened. Deep in the sulcus surrounding the nipple lies the projection of the "epithelial hood." Near the highest part of the nipple may be seen a slight excavation which represents the position of the future milk pore and intra-epidermal part of the primary duct. The lumina of the milk ducts are not completely formed, being best developed from the secondary or tertiary ducts to the free ends of the terminal ducts. As the primary duct is approached the lumen presents an irregular outline, while nearer the attached end of the duct it is represented by isolated lacunae. Near the surface there appears at this stage no trace of a lumen. From the secondary ducts to the end of the terminal ducts the epithelial walls are composed of two fairly definite layers of cells. From the secondary ducts toward the surface the cells gradually show an irregular arrangement until near its attached end the primary duct is represented by a solid cord of epithelial cells.

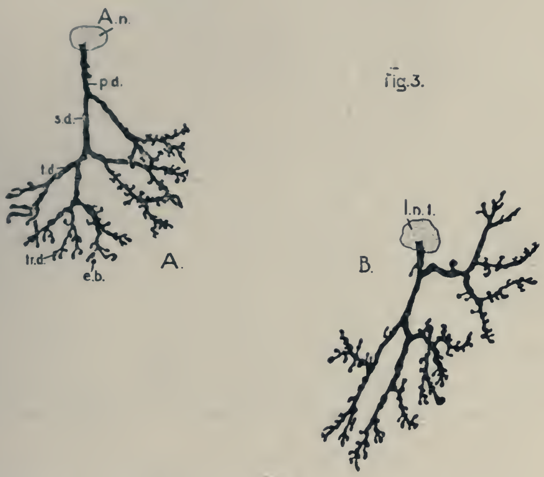


Fig. 3.—Same as Figure 1, but from a normal female of 3 weeks (weight 30 gm.), × 5.

---

[5] Myers, J. A.: The Histology of the Mammary Gland in the Male and Female Albino Rat from Birth to Ten Weeks of Age, Am. J. Anat. To be published (Abstr. in Anat. Rec. **14**:46, 1918.

Eight Days (Held at Birth Weight).—In a female that was held at birth weight for eight days the milk ducts (Fig. 2) show considerably less branching than those in a normal rat of seven days. They show, however, a slightly greater tendency to branch than the ducts of the normal new-born rat. The new branches are confined chiefly to the free end of the gland; however, an occasional collateral duct appears on the secondary and tertiary ducts.

When examined macroscopically the nipples appear more prominent than in the new-born. The nipple has, therefore, grown somewhat in spite of the fact that the gross body weight remains the same as at birth, or has even decreased.

Eighteen, Twenty and Twenty-One Days (Held at Birth Weight).—Figure 3 shows the milk ducts of the abdominal and first inguinal glands of a normal female albino rat of twenty-one days. When compared with Figure 1 it will be noticed that from birth to twenty-one days a considerable proliferation and growth of the ducts has taken place. Figure 4, drawn from a female albino rat held at birth weight for eighteen days, shows that the proliferation and growth of the milk ducts have been retarded to a marked extent. The ducts show very little or no development beyond those of the animal held at birth weight for eight days (Fig. 2), or the normal at birth (Fig. 1). The milk ducts in the male rat held at birth weight for eighteen days show approximately the same degree of retardation as those in the female. The ducts in a female held at birth weight for twenty days (Fig. 5) show about the same extent of development as those in the eighteen day stage.

Microscopic sections through the glands of a female show that in spite of the fact that the animal was held at birth weight the nipple has developed beyond the new-born stage. Its development has been considerably retarded, however, as it is much less prominent than the nipple of normal animals of corresponding age. The stimulus that causes the rapid growth of the nipple in normal rats about the second week has apparently been affected by the inanition, as the rapid growth of the gland at this stage has been inhibited. The nipple is considerably elevated above the surface (Fig. 12) of the surrounding epidermis. Partially surrounding the basal part of the nipple is the sulcus, deep in which may be seen the projection of the epithelial hood. This projection shows very little or no further development than appears in the new-born. The processes which appear on the projection of the normal animals of eighteen to twenty-one days (similar to those shown in Fig. 14) are very small in the starved rat. The external opening or milk pore appears near the highest part of the nipple, the lumen leading from this into the intra-epidermal part of the primary duct is quite shallow and is not continuous with the lumen of the primary duct proper. A cord of epithelial cells separates these two lumina, as is the case in normal animals until the second week of postnatal life is reached. The part of the attached end of the primary duct deep to the intra-epidermal portion is, therefore, without a lumen, but as one follows the sections toward the free end a lumen appears which is more regular in outline than in the new-born state, but is less well developed than in normal or control animals of same age as the test rats. Traced from the primary duct toward the free end of the system of ducts, the lumen presents the same general appearance as in normal animals of the same age except that it is somewhat smaller in caliber. The walls of the milk ducts beyond the primary duct possess the two layers of epithelial cells found in normal animals. Through the free end of the primary duct the cells are more irregularly arranged.

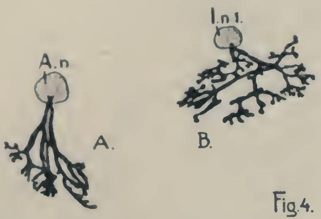


Fig. 4.—Same as Figure 1, but starved so as to weigh only 5 gm. at 18 days, × 5.

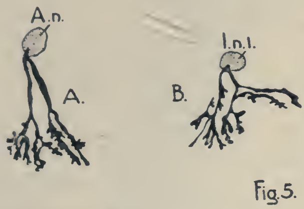


Fig. 5.—Same as Figure 1, but starved so as to weigh only 5 gm. at 20 days, × 5.

Thirty-Three Days (*Weight Restricted to 9.4 Gm.*).—The female control animal reached a weight of 49 gm. at the end of the thirty-third day. The milk ducts, as shown in Figure 6, have grown and proliferated very rapidly since the 21-day stage (Fig. 3). While there is no overlapping between the ducts of adjacent glands, the ducts of the second thoracic gland are very nearly in apposition with those of the first and third thoracic glands. As seen in Figure 6, the ducts of the abdominal and first inguinal glands very nearly meet. Only a small interspace exists between the ducts of the first and second

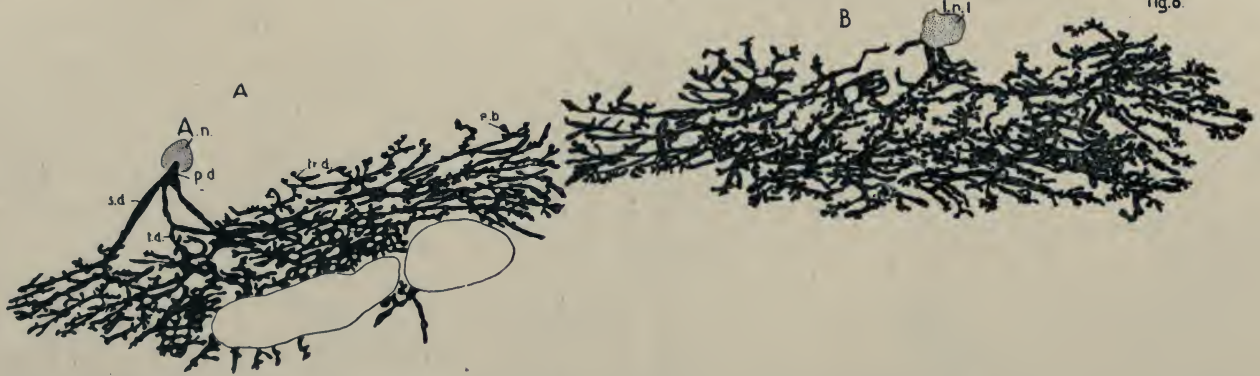


Fig. 6

Fig. 6.—Drawn from a cleared preparation (internal view) of a normal female albino rat of 33 days (weight 49 gm.) to show distribution and relations of ducts of right abdominal gland (A), and right first inguinal gland (B), ×5 A.n., abdominal nipple; I.n.1, first inguinal nipple; p.d., primary duct; s.d., secondary duct; t.d., tertiary duct; tr.d., terminal duct; e.b., end-bud; L., lymph node.

inguinal glands. An examination of the glands of the underfed test animal, whose body weight never exceeded 9.4 gm., reveals the fact that the milk ducts are still in a very primitive state of development (Fig. 7). In spite of the fact that the body weight of this animal was allowed slightly to exceed that of a normal animal of one week, the milk ducts do not show as extensive proliferation and growth as those in a normal animal of one week. In fact some of the glands of this animal appear very slightly or no better developed than those in normal animals at birth.

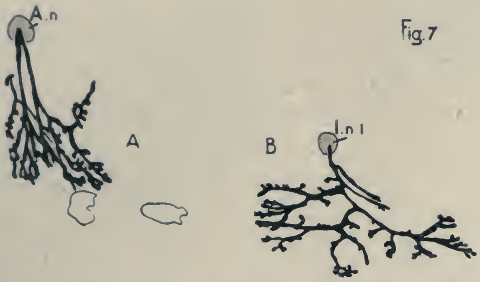


Fig. 7. Same as Figure 6 but from test animal of same litter which was starved so as to weigh only 9.4 gm. at 33 days, × 5

The study of microscopic sections shows the nipple slightly more elevated above the surface of the surrounding integument than in test animals of 18 to 21 days of age. The sulcus around the nipple presents approximately the same depth as in the preceding stage. The projection of the "epithelial hood" is in a rudimentary state of development. The sections show rather numerous projections extending inward from the epidermis covering the nipple. The lumen of the intra-epidermal part of the primary duct has deepened somewhat, but is not quite continuous with that of the primary duct proper.

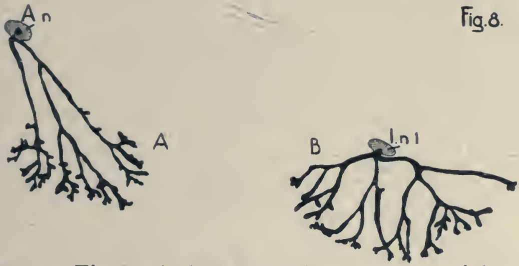


Fig. 8.—Same as Figure 1, but starved so as to weigh only 10 gm. at 38 days, × 5.

Thirty-Eight Days (*Weight Restricted to 10 gm.*).—In a female of 38 days, whose body weight never exceeded 10 gm., the milk ducts show a very slight increase in length over those in the normal newborn. In Figure 8 it will be observed that there is an occasional collateral duct branching from the secondary and tertiary ducts. Generally, however, collateral ducts are not numerous on any part of a system of ducts. Compare this and preceding with normal at 1 week (about 10 gm.).

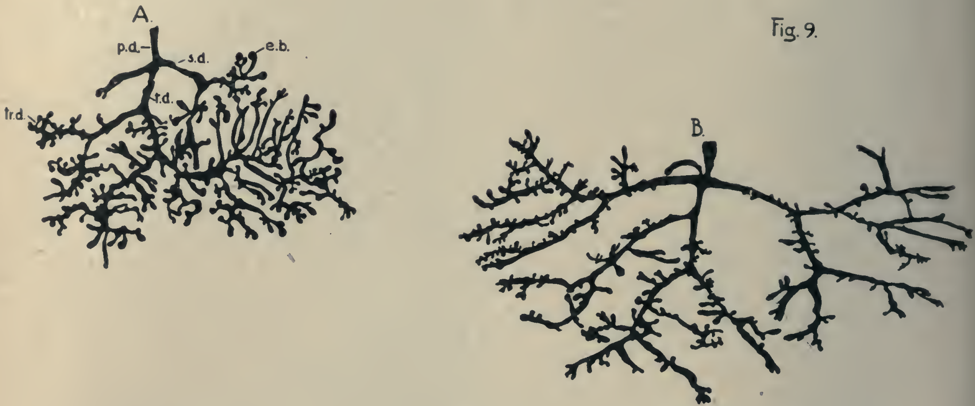


Fig. 9.

Fig. 9.—Drawn from a cleared preparation (internal view of a male albino rat of 58 days, weight 96 gm.) to show distribution and relations of ducts of right abdominal gland (A), and right first inguinal gland (B), × 5. In the illustration p.d. = primary duct; s.d., secondary duct; t.d., tertiary duct; tr.d., terminal duct; e.b., end-bud; L., lymph note.

Fifty-Eight Days (Weight Restricted to 10.9 gm.).—The male control animal weighed 96 gm. at the age of 58 days. The milk ducts (Fig. 9) show about the normal amount of branching for males of this age, there being a failure of the ducts to respond to the puberty changes occurring in the female.[1] In the test animal with a body weight of 10.9 gm. the milk ducts (Fig. 10) show approximately the same degree of retardation as those of the female. In other words, the ducts show about the same number of branches, and are, in general, of the same size as at the time of birth, notwithstanding the fact that the body weight of the test animal is more than double that of the new-born.

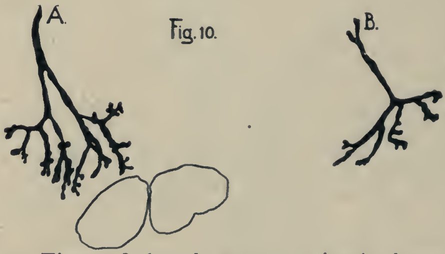


Fig. 10.

Fig. 10.—Same as Figure 9, but from test animal of same litter which was starved so as to weigh only 10.9 gm. at 58 days, × 5.

Fifty-Eight Days (Weight Restricted to 15.5 Gm.).—This animal was allowed to reach a body weight equal to that of a normal rat of 2 weeks. The increase in weight over the last stage, however, had no apparent effect on the growth or proliferation of the milk ducts, as they do not appear noticeably different from those of an animal of the same age with a smaller body weight.

The nipples in microscopic sections show no greater elevation than in animals held at 9.4 gm. for thirty-three days. Normal rats with a body weight of 15.5 gm. possess nipples much more elevated than underfed animals of the same weight. It appears, therefore, that during underfeeding very little of the weight added to the body between the 33 and 58-day stages is accompanied by growth of the mammary glands. The systems of milk ducts in the 58-day underfed rats show the same structure as earlier described stages. The lumina do not communicate with the exterior, owing to the cord of epithelial cells persisting between the lumen of the intra-epidermal part of the primary duct and that of the primary duct proper. The lumen of the intra-epidermal part of the primary duct contains débris derived from the process of desquamation and delamination in the formation of the lumen.

Sixty-Five Days (Weight Restricted to 8.5 Gm.).—It was shown earlier[4] that the milk ducts of the normal female albino rat undergo a tremendous proliferation during the eighth or ninth week — the time when puberty usually appears. The control animal used in the present study had reached a body weight of 82.5 gm. on the sixty-fifth day, the time of killing. The ducts of the mammary glands have developed to such an extent that there is a decided overlapping of the ducts of adjacent glands. The ducts of the thoracic glands have overlapped so as to form on each side a continuous arborization of ducts which extends from the cephalic portion of the first thoracic to the caudal part of the third thoracic gland. The ducts of the abdominal and inguinal glands on each side likewise show a continuous arborization which extends from the cephalic part of the abdominal to the most caudal ducts of the second inguinal gland. In the test animal, which was held at 8.5 gm. by underfeeding, no such development has occurred. The milk ducts are somewhat longer than those in the new-born, but otherwise there is no apparent difference.

Microscopic sections through the nipples of test and control animals show a considerable contrast. The nipple of the control has reached a considerable elevation and has taken on a somewhat conical form. The projection of the "epithelial hood" presents numerous processes, while in the central part of the attached end of the projection degenerative changes are in progress. The system of milk ducts communicates freely with the exterior through the milk pore. The nipple of the test rat, on the other hand, is only slightly elevated and does not present a conical form. The projection of the "epithelial hood" is very rudimentary. There is a very slight communication of the system of milk ducts through the milk pore, the lumen of the intra-epidermal part of the primary duct not being completely formed.

Seventy Days (*Weight Restricted to 16.5 Gm.*).—The milk ducts of this female rat show nothing different from that which has been described in the foregoing stages. The ducts remain in a very poorly developed state.

Fig. 11.

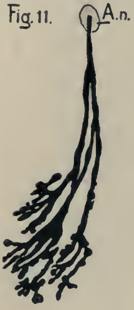


Fig. 12.

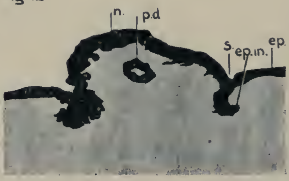


Fig. 13

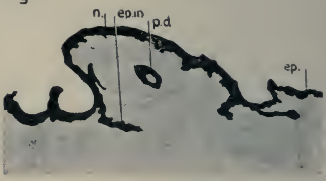


Fig. 11.—Same as Figure 1, but from a test animal which was starved so as to weigh only 8.5 gm. at 65 days, × 5.

Fig. 12.—Drawn from a section through the nipple of a female albino rat. Starved so as to weigh only 5 gm. at 18 days, × 66⅔; ep., epithelium; ep.in., epithelial ingrowth or hood; n., nipple; p.d., primary duct; s., sulcus around base of nipple.

Fig. 13.—Drawn from a section through the nipple of a female albino rat. Starved so as to weigh only 16.5 gm. at 70 days, × 66⅔. Compare with Figure 14. ep., epithelium; ep.in., epithelial ingrowth or hood; n., nipple; p.d., primary duct; s., sulcus around base of nipple.

Microscopic sections (Fig. 13) show the nipple remaining at about the same degree of elevation as in the 33-day underfed rats. The projections of the "epithelial hood" have failed to develop beyond the stages previously described. A slight communication has appeared between the lumen of the intra-epidermal part of the primary duct and that of the primary duct proper. Therefore, the lumina of the system of ducts have a common opening to the exterior through the milk pore. This condition normally is present in the rat at the end of the second week of postnatal life; however, it is usually not until the fifth or sixth week that this communication has attained its normal caliber. The two-layered arrangement of the epithelial walls of the milk ducts persists. No parts resembling developing alveoli have been observed, and there is no indication of lobulation. The mantle layer (which immediately surrounds the milk ducts) of the stroma is thinner than in normal rats of the same age. The true stroma which normally contains such an abundance of fat is nearly or quite free from fat. The entire stroma appears much more cellular and less vascular than in normal animals of the same age.

2. *Effects of Refeeding.*—This part of the present article should be regarded only as a preliminary report, as further work now in progress will be presented at a later time.

Fig 14

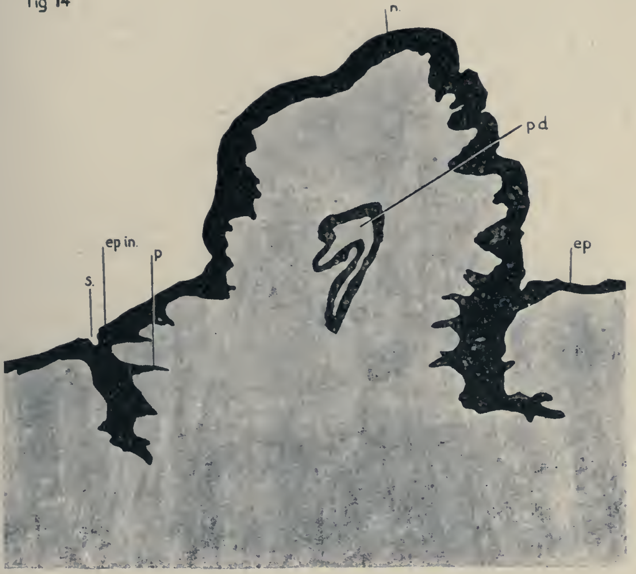


Fig. 14.—Drawn from a section through the nipple of a normal female albino rat of nine weeks, × 66⅔. Labeling same as in Figure 13. Compare with Figure 13.

Seventy days (weight restricted to 10 gm. for nineteen days, then placed on normal diet. Weight 42 gm.): The glands of the control animal show the normal extensive development which occurs about the time of puberty described in an earlier article.[4] From the gross body weight of the test rat one might expect to find the milk ducts in a stage of development nearly equal to those of a normal rat of 4 or 5 weeks. This, however, is not the case. As shown in Figure 15, the ducts have grown and proliferated considerably beyond those in animals of 18 to 21 days held at approximately birth weight (Figs. 4 and 5). The ducts of this animal are quite comparable in their extent of development with those of a normal rat of about 3 weeks (compare with Fig. 3).

Microscopic sections show that the nipple in the refed animals possesses a diameter somewhat greater than that of the underfed animal of 70 days. The elevation is approximately the same. The refeeding has apparently had an effect on the development of the projection of the "epithelial hood," as this projection now presents a somewhat greater diameter than in animals before the refeeding; besides, it possesses a number of processes, as does also the inner surface of the epidermis covering the nipple. These processes are somewhat larger than those seen in the starved rats (Figs. 12 and 13).

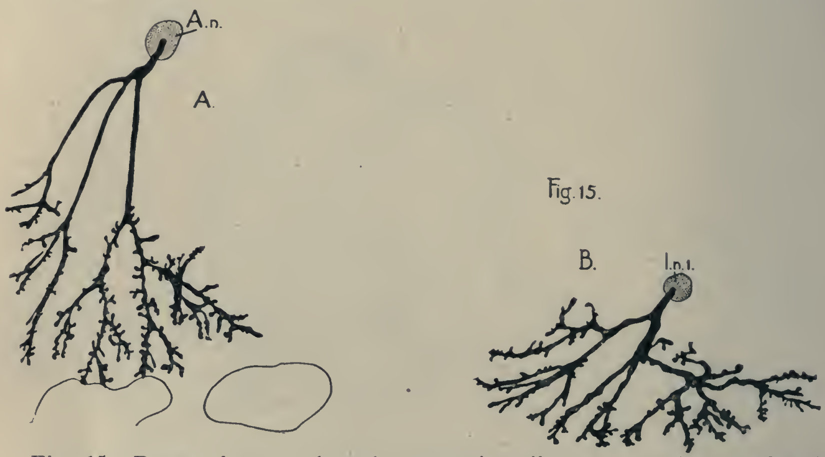


Fig. 15.—Drawn from a cleared preparation (internal view) of a female albino rat to show distribution and relations of ducts of right abdominal gland (A), and first inguinal gland (B), × 5. This animal was starved so as to weigh only 10 gm. at the nineteenth day of life, after which it was placed on normal diet until 70 days old, when it weighed 42 gm. A.n., abdominal nipple; I.n.1, first inguinal nipple; p. d., primary duct; s.d., secondary duct; t.d., tertiary duct; tr.d., terminal duct; e.b., end-bud.

The milk ducts have increased somewhat in diameter, but the epithelium of the walls is arranged in the two usual layers except the intra-epidermal part of the primary duct, which is lined with the stratified squamous type. The epithelial cells appear somewhat larger than in animals that have not been refed and mitotic figures are not uncommon. The stroma does not appear as cellular as when the animals were being starved. Moreover, a considerable amount of fat has made its appearance in the true stroma.

One hundred and twelve days (weight restricted to 10 gm. for nineteen days; then placed on normal diet until gross body weight reached 85 gm.; that of control killed at 70 days): This female rat was from same litter as the preceding. Figure 16 shows that the milk-ducts have undergone a considerable development beyond those of the sister killed at 70 days, with a body weight of 42 gm. They have not, however, reached the same degree of development as those of the control killed at 70 days with a corresponding body weight. In the control animal, the ducts of the thoracic glands overlap so as to form a continuous arborization on each side. The abdominal and inguinal glands also form on each side a continuous mass of gland tissue. In the test rat, the ducts of the abdominal and inguinal glands are nearly in apposition and only rarely is there an overlapping. Collateral ducts are much more numerous on the secondary, tertiary and quaternary ducts than on those milk ducts nearer the free end of a system. In many cases the collateral ducts present many branches. The milk ducts as a whole appear to have a diameter smaller than those of normal animals and the terminal end-buds are not so numerous. Those present, however, stand out very strikingly (Fig. 16).

One hundred and twenty-five days (weight restricted to 10 gm. for twenty days; then placed on normal diet until time of killing. Gross body weight 97 gm.): In this female specimen the ducts show an arborization very similar to that of a normal rat of about the age of puberty. Several individuals subjected to similar treatment and killed at approximately the same age as this animal show the milk ducts in a condition similar to those of this specimen. Therefore, we can conclude that in animals subjected to severe inanition during the first three weeks of life the mammary glands are so retarded that it is not until near the eighteenth week that the glands have attained a development equal to that of normal animals of about 9 weeks. Along with the growth of the milk ducts the nipples have developed so that they, too, have reached a stage similar to that reached in normal animals about the age of puberty.

Similar refeeding experiments have been carried out on male individuals with results very similar to those obtained in females except that the male ducts do not reach such a high stage of development. The ducts are retarded in the male as in the female; on refeeding, however, they apparently only keep pace with the growth of the body of the animal.

DISCUSSION AND CONCLUSIONS

The test rats used in the first part of this paper may be divided into three classes. In the first class we have those animals that were held at birth weight and killed at various intervals up to about 3 weeks of age. In the second class are included animals which were starved from birth but were allowed to reach a weight approximately double that at the time of birth. These animals were killed at various stages, the oldest being 65 days of age. The third stage includes animals starved from birth, but whose body weights were allowed to increase

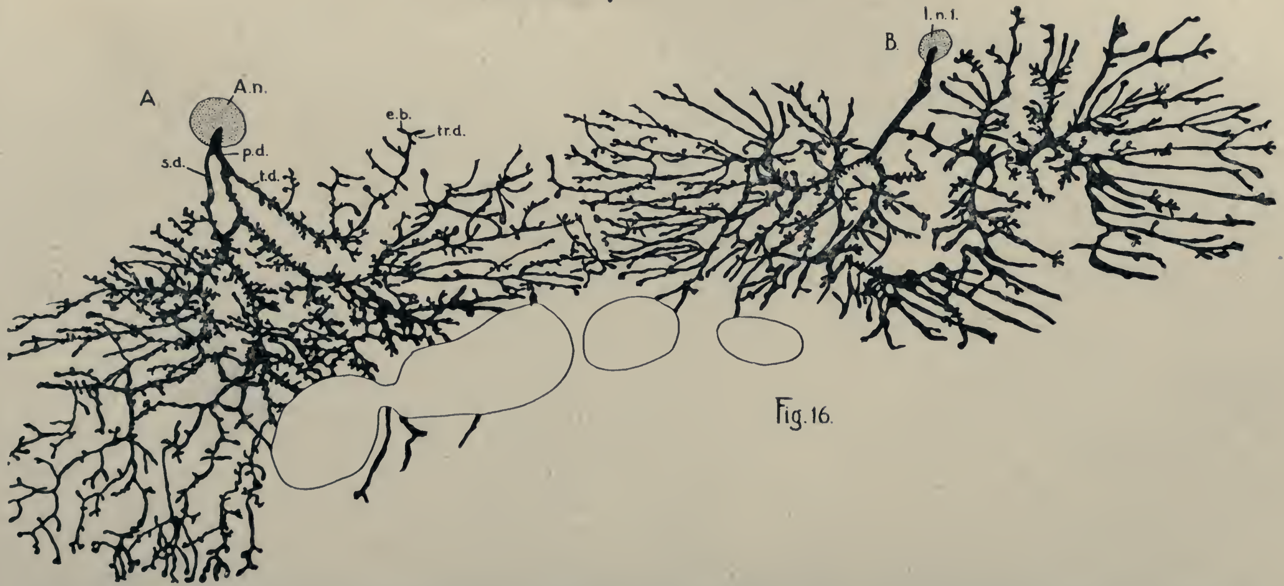


Fig. 16.

Fig. 16.—Same as Figure 15, but animal was placed on normal diet at end of period of starvation until 112 days old, at which time it weighed 85 gm., × 5. Compare this figure with Figures 12 and 13 of an earlier paper (Am. J. Anat. **19**:385, 1916.

to approximately three or four times the birth weight. The oldest animal in this class was underfed for seventy days before killing.

In the first class it was noticed that the milk ducts grew very little during the first week, after which time growth apparently ceases. However, the nipple becomes somewhat more elevated above the surface than normally at the time of birth.

It has been shown (Stewart[6]) that in animals held at birth weight by underfeeding for various periods, the integument increases about 25 per cent. in weight. There is normally a strong tendency for the integument to grow during the first week of life. Jackson and Lowrey[7] and Jackson[8] showed that during this first week of life the integument increases from 20 per cent. to 26 per cent. of the body weight. In Stewart's test animals a fair coat of hair developed. It is therefore possible that the hair and follicles play a considerable rôle in increasing the weight of the integument. As shown in the present article, the mammary glands increase in size but little, however, and therefore scarcely participate in the increase of the weight of the integument during the underfeeding period. Further histologic study of the integument is necessary to ascertain whether parts other than the hair and mammary glands increase in size during this severe inanition.

In the second class, where the body weight in the underfed rats doubled that at the time of birth, the milk ducts show a slight increase, but are not equal to those of a normal animal of the same body weight. These animals are considerably older than those in Class 1, all being killed after the third week. In rats similarly underfed from birth, the body weight being retarded so as to reach only 10 gm. at 3 weeks of age, Stewart[9] found the weight of the integument about equal to that of normal controls of the same body weight. In rats underfed at a later period, beginning at three weeks of age, Jackson[10] has shown that there is a marked loss in the relative weight of the integument.

---

[6] Stewart, C. A.: Changes in the Weights of the Various Parts, Systems and Organs in Albino Rats Kept at Birth Weight for Various Periods, Am. J. Physiol. **48**:67, 1919.

[7] Jackson, C. M., and Lowrey, L. G.: On the Relative Growth of the Component Parts (Head, Trunk and Extremities) and Systems (Skin, Skeleton, Musculature and Viscera) of the Albino Rat, Anat. Rec. **6**:449, 1912.

[8] Jackson, C. M.: Postnatal Growth and Variability of the Body of the Various Organs in the Albino Rat, Am. J. Anat. **15**:1, 1913.

[9] Stewart, C. A.: Changes in the Relative Weights of the Various Parts, Systems and Organs of Young Albino Rats Underfed for Various Periods, J. Exper. Zool. **25**:301, 1918.

[10] Jackson, C. M.: Effects of Acute and Chronic Inanition on the Relative Weights of the Various Organs and Systems of Adult Albino Rats, Am. J. Anat. **118**:75, 1915; Changes in the Relative Weights of the Various Parts, Systems and Organs of Young Albino Rats Held at Constant Body Weights by Underfeeding for Various Periods, J. Exper. Zool. **19**:99, 1915.

The effect of inanition at various ages on the weight of the integument is discussed by Jackson[10] and Stewart.[11] They state that the loss in weight of the integument in young rats during underfeeding is probably due largely to loss of fat.

In a study (uncompleted) on the subcutaneous fat of the albino rat I have been able to demonstrate the presence of considerable amounts of such fat deposit in fairly definite pads in the subcutaneous tissues of the rat before three weeks of age. In the underfed rats used by Jackson and Stewart the fat deposited in the subcutaneous fat depots was of course soon consumed. In my preparations the starving began at the time of birth, but extended far beyond the three week stage. In such rats the body weight increased somewhat during which time the mammary glands showed a very slight increase. No observations have been made to determine whether the mammary glands remain stationary or (like the weight of the integument) actually undergo a decrease in size when the inanition begins at the third week of age or later.

The mammary glands of the rats in the third class (although subjected to less severe inanition) do not show anything essentially different from those of the second class just described. In general, the development of the mammary glands in rats thus stunted by underfeeding is retarded in proportion to the body weight.

It is generally believed that there is such a close relationship existing between the mammary glands and the ovaries that when important changes take place in the ovaries the mammary gland might also be affected. In view of this fact it is interesting to compare the growth of these organs in starved animals. Stewart[6] showed that in animals held at birth weight for sixteen days the ovaries increase about 5 per cent. The mammary glands from these same animals here described under Class 1 show only a very slight increase. Therefore, one may conclude that the two organs undergo a somewhat parallel development under these conditions. In those animals underfed from birth to 3 weeks, body weight 10 gm., and those underfed from birth to 10 weeks, body weight 15 gm., Stewart[9] found the ovaries increased in weight 83 per cent. and 54 per cent., respectively. My Figures 7 and 8 show a much smaller increase in the mammary glands under the same conditions. There is, therefore, apparently no close correlation between the growth of the mammary glands and that of the ovaries in underfed young rats.

As shown by the present study, the mammary glands apparently respond somewhat slowly when the animals are refed after under-

---

11. Stewart, C. A.: Growth of the Body and of the Various Organs of Young Albino Rats after Inanition for Various Periods, Biol. Bull. **31**:16, 1916.

feeding from birth to about 3 weeks of age. In fact, the response was so delayed that the mammary glands did not reach the stage of development ordinarily attained at the time of puberty (about 8 or 9 weeks) until the refed rat had reached an age of about 18 weeks. Stewart[11] showed that in rats on which the underfeeding was begun at the time of weaning (3 weeks) or later, and underfed for short periods, the integument and ovaries recovered very rapidly when the animals were refed. Later, however, Jackson and Stewart[9] showed that when the underfeeding is begun at birth and continued three weeks or longer, a permanent stunting of the body usually occurs. In view of the great individual variation in the development of the mammary glands at any given age or body weight (as shown by Myers[4]), it is hazardous to draw any final conclusions from the relatively few cases observed in the present study. Apparently, however, the mammary glands in the underfed young rats, though temporarily lagging somewhat behind the body weight when the rats are fully refed, ultimately may attain a normal degree of development.

SUMMARY

1. Severe inanition retards the growth of the milk ducts of the female rat during the first week, but apparently does not completely stop their growth. In animals held at birth weight for a longer time the ducts cease to grow, and remain in a condition slightly more developed than at the time of birth. If after the first week the gross body weight of the animals is allowed to increase so as to correspond with that of a normal animal of one week, the milk ducts fail to develop to the same extent as those of a normal animal of corresponding body weight. This also holds true if the body weight of the underfed rat is allowed to equal that of a normal animal of two weeks.

2. The lumen of the primary duct in underfed rats does not communicate with the exterior through the milk pore until the tenth week.

3. The growth of the milk ducts of male rats is retarded by inanition in a manner similar to that of the female.

4. The nipple grows very little during inanition, being elevated above the surface only slightly in young rats starved severely for eight to ten weeks. The epithelial processes fail to develop much beyond the stage reached at birth, and the sulcus around the base of the normal nipple remains shallow.

5. The subcutaneous fat that appears very early in the neighborhood of the milk ducts soon becomes greatly decreased after the amount of food is reduced to a minimum.

6. In all, the retardation in the development of the mammary gland is roughly proportional to the retardation in body weight (at least within limits of normal variability).

7. Severe inanition for a short time at an early age thus temporarily stunts the mammary glands. When the animal is refed the glands respond slowly. When the body weight during refeeding reaches that of a normal rat at the age of puberty, the milk ducts are far behind those of the normal rat at corresponding body weight. That this stunting is not permanent is shown by the fact that the ducts ultimately attain the same stage of development as those of a normal animal, but at a much later period.

Reprinted from the American Journal of Diseases of Children
May, 1919, Vol. XVII, pp. 311-328

76.9. 11

STUDIES ON THE MAMMARY GLAND

VI. THE DEVELOPMENT OF THE MAMMARY GLAND FROM ITS EARLIEST APPEARANCE UNTIL THE PERIOD OF PREGNANCY *

J. A. MYERS, M.S., Ph.D.
MINNEAPOLIS

The development of the various parts of the mammary gland will be discussed in the following order: Mammary streak, mammary line, mammary hillocks, milk ducts, formation of lumen, new-born secretion, puberty changes, changes from puberty to the first pregnancy, the stroma, the nipple, supernumerary mammary glands, effects of inanition on the developing mammary glands and comparison of the developing mammary glands in the two sexes.

Mammary Streak, Mammary Line and Mammary Hillocks.—The mammary gland makes its first appearance quite early in the embryonic life of various species of animals. In the albino rat Henneberg[1] observed the first trace of the mammary gland in embryos of eleven days of gestation. Lustig[2] described the mammary gland as first appearing in human embryos of 4.75 mm. in length. The mammary gland in each species above mentioned is first represented on each side of the embryo by a faint light streak which extends from the anterior limb bud near the region of the future axilla to the posterior limb bud near the inguinal region. This streak, which has been designated the mammary streak, is uniform in thickness throughout its entire extent. Making a cross section through this streak and examining it microscopically, it may be observed that, like the adjacent epidermis, this streak is composed of a single layer of cells. The cells of the streak, however, are slightly larger and somewhat more elongated than those of the adjacent epidermis.

Very soon the cells of the mammary streak begin to proliferate and this process continues until several layers of cells are present. By this time the original faint streak has been converted into a very definite line, the mammary line, which is considerably elevated above the surface of the epidermis and occupies the same position as the earlier mammary streak.

---

* From the Institute of Anatomy, University of Minnesota.

* Read before the Northwestern Pediatric Society, Minneapolis, Feb. 13, 1919.

[1] Henneberg, Brunno: Die erste Entwickelung der Mammarorgane bei der Ratte, Anat . Hefte **13**: 1900.

[2] Lustig, Hilda: Zur Entwicklungsgeschichte der menschlichen Brustdrüse, Arch. f. mikr. Anat. **87**: 1916.

In the mammary line, unlike the mammary streak, the proliferation of cells and growth of the various parts is so unequal that there soon appear in the mammary line of each side outstanding elevations which have been designated mammary hillocks. The number of hillocks which appear depends largely on the number of glands found in the adult of any given species. As the hillocks develop, the parts of the mammary line intervening between them gradually disappear leaving only the isolated hillocks to represent the future mammary glands. The hillocks are somewhat biconcave in shape with the greater convexity resting on the subjacent mesenchyma. As development progresses the hillocks sink deeper and deeper into the mesenchyma until their superficial surface is on a level with the surrounding epidermis. That part embedded in the mesenchyma becomes a somewhat oblong or spherical mass of epithelial cells attached to the surface epidermis only by a rather constricted neck.

The Milk Ducts.—Early History of the Ducts: From the deep surface of the spherical masses of epithelial cells above described there develop small solid epithelial buds. Each bud represents a future milk duct and consequently the number of buds present at this stage corresponds with the number of milk ducts characteristic of the species. In man, for example, fifteen to twenty buds develop from the deep surface of each spherical mass of epithelial cells, while in the albino rat only one such bud develops from each mass. Each one of these primary solid epithelial buds pushes its way into the mesenchyma and soon divides into two secondary buds. The secondary buds likewise push into the mesenchyma and divide into tertiary buds. This dichotomous method of division continues until large numbers of milk ducts are present. Not all of the later divisions are by the dichotomous method.

Formation of the Lumen.—The milk ducts in most species of animals that have been studied present a complete or partial lumen sometime before birth. De Sinety[3] and Lustig[2] found the lumen making its first appearance in human fetuses of about the sixth or seventh month. In the cat and one species of bat (*Vespertilio murinis*) Brouha[4] described the lumen as being partly developed in the late fetal stages or soon after birth. In the albino rat the lumen first appears in fetuses of eighteen or nineteen days and is fairly well developed in most parts of ducts at the time of birth.[5] Earlier investigators, as De Sinety[3] and Rein,[6] believed that the lumina of the milk ducts are formed by the process of degeneration. On the contrary, however, the later works of Benda,[7] Brouha[4] and Myers[5] show that the lumen of the ducts of at least some species of animals is formed by the process of rearrangement of cells.

---

[3] De Sinety: Recherches sur la mamelle des enfants nouveaunes, Arch. de physiol, norm. et patholog. **2**: 1875.

[4] Brouha: Recherches sur les diverses phases du developpement et de l'activite de la mamélle, Arch. de Biol. **21**: 1905.

Newborn Secretion (Witches' Milk).—At the time of birth the milk ducts show a considerable branching, as many as six or seven divisions being present in some individuals. Soon after birth a slight secretion appears in the milk ducts of human infants. This secretion increases in amount so that about the third day it can be expressed from the nipple of the infant. It may accumulate in such quantities as to engorge and distend the ducts, thus producing an enlargement of the gland which, when viewed from the surface, may appear as large as a half walnut. Ordinarily, the secretion ceases by the twentieth day after which the secretion already present gradually disappears, being carried away by the leukocytes or absorbed directly; however, a few cases have been reported in which the secretion persisted for at least three months.

Through the kindness of Prof. J. P. Sedgwick I have been permitted to make a considerable number of observations on infants at the University of Minnesota hospitals. Such observations have shown that the new-born secretion may be present in some infants at the time of birth; such infants, however, usually show indications of having been born postmaturely. On the other hand, in premature infants the secretion may not appear until many days after birth, and in a few cases it was not observed at all.

It has been proved quite conclusively that the new-born secretion contains all the constituents of true milk. The question therefore arises as to whether the stimulus which brings on the activity of the mammary glands of the mother is the same one that excited the glands of the new-born to secrete witches' milk. For a long time it was believed that the exciting factor was some substance produced and liberated from the placenta at the time of delivery and that it entered both maternal and fetal circulations thus causing the activity of the mammary glands of both mother and offspring. Later, however,

---

[5] Myers, J. A.: Studies on the Mammary Gland. II. The Fetal Development of the Mammary Gland in the Female Albino Rat, Am. J. Anat. **22**: 1917. (Abstr. published in Anat. Rec. **11**:390, 1917.)

[6] Rein, G.: Untersuchungen über die embryonale Entwicklungsgeschichte der Milchdrüse, Arch. f. mikr. Anat. **20**: **21**: 1882.

[7] Benda, L.: Das Verhältnis der Milchdrüsen zu den Hautdrüsen, Dermat. Ztschr. **1**: 1894.

Brouha[4] and Schil[8] showed that in well developed infants the new-born secretion may completely precede the birth of the child. From these observations it would seem that a secretion capable of stimulating the glands to action is formed in the placenta or ovary of the mother and transmitted to the blood of both mother and offspring. This may sufficiently explain the source of the stimulus in those infants whose glands secrete before or immediately after birth, but what explanation shall we offer for those that are born prematurely and whose glands do not show any appreciable amount of secretion for many days or even weeks after birth. There is in these cases the possibility that the substance which stimulates the glands of the mother may be transmitted to the offspring through the mother's milk. It is probable that a series of observations on children that have never taken mother's milk may throw some light on this subject.

My observations on the mammary glands of new-born albino rats[9] showed that the milk ducts may contain a slight amount of secretion a few days after birth. Specific fat stains, however, revealed the fact that no fat appears in the lumen of the ducts, therefore this substance cannot be called a true milk secretion.

Brouha,[5] after studying the young of one species of bat, the rabbit and the domestic cat concluded that a slight secretion appears in the milk ducts but does not become a true milk secretion at any time during the first three weeks of life. This failure of the mammary glands of the new-born of some of the lower animals to produce true milk may be due to the feeble development of the glands at the time of birth, as the offspring of some of these species are born in a very immature state and consequently the mammary glands are not as well developed as in the human new-born. On the other hand, the question arises as to whether the stimulus which produces a true lactation in the human new-born is actually present in some lower forms of animals. Further experimental evidence is necessary to decide this question.

After the secretion of the new-born disappears the ducts resume a state similar to that at the time of birth. For some time after birth the milk ducts undergo a very gradual growth.

Puberty Changes.—As the time of puberty approaches, the milk ducts begin to proliferate and grow very rapidly, resulting in a dense

---

8. Schil, L.: Recherches sur la glande mammaire, sur les phases qu'elle présente au cours de son évolution et leur determinisme, Thèse, Lyon, 1912.

9. Myers, J. A.: The Histology of the Mammary Gland in the Male and Female Albino Rat from Birth to Ten Weeks of Age, Am. J. Anat., July, 1919. (Abstr. published in Anat. Rec. **14**:46, 1919.)

arborization of ducts. It was shown[10] that this rapid development occurs in the albino rat about the eighth or ninth week—the time when puberty normally appears. It was observed that these changes in the milk ducts correspond with marked changes in the ovaries which have become highly vascular and show large Graafian follicles in the process of development. That similar changes occur in the human mammary glands at the time of puberty is beyond question. Here one can observe from external examination that the glands become much more voluminous as the period of puberty approaches. That the increase in size is not entirely due to the deposition of fat in this region is proved by the fact that the corpus mammae which at this time is palpable has increased noticeably in size, also necropsy findings show that there is a tremendous proliferation of the gland parenchyma shortly before and at puberty.

Changes from Puberty to the First Pregnancy.—If pregnancy does not occur with the first ovulation the mammary glands undergo slight retrogressive changes; however, as the next ovulation approaches the mammary gland takes on a new development. Similar changes can be observed with each succeeding ovulation until pregnancy occurs. The changes in the mammary gland during ovulation are so marked in some species of animals that one is able to tell when the animal is in heat simply by palpating the mammary gland. Hartman[11] has taken advantage of this fact in collecting material for embryologic work on the marsupials. It has long been known that there is an enlargement and tenderness of the mammary glands in women preceding and during the menstrual period.

The Stroma.—As the solid epithelial buds representing the future milk ducts press their way into the mesenchyma they carry with them a thin layer of mesenchyma which later develops into the mantle layer. The remaining mesenchyma in this region later develops into the true stroma. Elastic tissue fibers can be observed in the sheaths of the milk ducts in the albino rat after the third week of extrauterine life. Berka[12] made a careful study of the elastic tissue in the human mammary gland.

---

[10] Myers, J. A.: Studies on the Mammary Gland. I. The Growth and Distribution of the Milk Ducts and the Development of the Nipple in the Albino Rat from Birth to Ten Weeks of Age, Am. J. Anat. **19**: 1916. (Abstr. published in Anat. Rec. **10**:230, 1916.)

[11] Hartman, Carl D.: Studies in the development of the opossum (Didelphys virginiana L.). III. Description of new material on maturation, cleavage, and entoderm formation, 1919, J. Morphol. **32**: 1919.

[12] Berka, F.: Die Brustdrüse verschiedener Altersstufen und während der Schwangerschaft, Frankfurter Ztschr. f. Pathol. **8**: 1911.

Near the time of birth in the albino rat a marked infiltration of fat begins to appear in the regions already occupied or that will later be occupied by the milk ducts.[13] This fat is at first deposited in definite pads, one of which, the inguinal fat pad, receives the ducts of the abdominal and the two inguinal glands. The thoracic fat pad receives the milk ducts of the second and third thoracic glands while the ducts of the first thoracic gland ramify in the cervical pad of fat. The pads of fat at first lie lateral and dorsal to all parts of the glands except the terminal ducts. The ducts grow laterally and the pads of fat while increasing in size extend medially until the ducts of the mammary glands are completely imbedded in fat. In this connection it is interesting to note that Berg[14] also found in human embryos that the subcutaneous fat first appears in the thoracic and inguinal region. One must not lose sight of the fact that the subcutaneous blood supply is quite rich in the regions where the mammary glands develop. The question therefore arises as to whether the deposit of fat is influenced by the mammary glands or whether it happens to be laid down here on account of the vascularity of the region. Again, one might ask if the fat is deposited in the region of the mammary glands because it is a good medium for the milk ducts to proliferate in or does it have some special rôle to play in the later functional stages of the milk ducts during pregnancy and lactation.

The Nipple. — Returning to the mammary line, which in early embryos extends from the anterior limb bud to the posterior limb bud, we find that through unequal growth certain parts of the line become quite prominent while the intervening parts of the line disappear, leaving the isolated prominent masses — the mammary hillocks. These hillocks occupy the positions which the future nipples will occupy and consequently their number corresponds with that of the future nipples.

As the mammary hillock sinks into the mesenchyma and its superficial surface becomes level with the surface of the surrounding epidermis an excavation begins to be formed through the processes of degeneration and desquamation. This process continues until there is developed a fair sized pit — the mammary pit. Very soon one observes at the bottom of this pit a small papilla-like elevation — the anlage of the nipple. The nipple anlage continues to grow toward the surface until it fills the mammary pit. At the time of birth in the albino rat the nipple is usually about on a level with the surface of the surrounding epidermis. My unpublished observations made at the University of Minnesota Hospital show that in many cases the nipples of the human new-born have not yet reached the surface of the surrounding epidermis, in others the nipples just fill the mammary pits while in the remainder of cases the nipple is slightly elevated above the surface of the skin. After birth the nipples in most individuals become elevated above the surface and show a very gradual growth until the time of puberty, when they take on their adult size and form. In the human each of the fifteen or twenty milk ducts has a separate opening — milk pore — on the surface of the nipple, while in the albino rat there is a single milk pore.

---

[13] Myers, J. A., and Myers, Frank J.: Studies on the Mammary Gland. VII. The Distribution of the Subcutaneous Fat and Its Relation to the Developing Mammary Glands in Male and Female Albino Rats from Birth to Ten Weeks of Age, Abstr. in Anat. Rec., May, 1919.

[14] Berg, W.: Ueber die Anlage und Entwickelung des Fettgewebes beim Menschen, Ztschr. f. Morphol. u. Anthropol. **13**: 1911.

Supernumerary Mammary Glands. — We have already seen that the mammary glands develop in the mammary line from the points where mammary hillocks appeared. Ordinarily, each species develops a fairly constant number of glands. In some animals, the pig for example, there is a row of mammary glands on each side of the ventral body wall extending from the axillary to the inguinal region. In the sheep, cow and other animals, mammary glands usually appear only in the inguinal region, while in man the glands ordinarily appear as a single pair in the thoracic region. Now those animals, including man, that present glands only in a certain part of the old mammary line region, have in the embryonic stage a well developed mammary streak and line, on each side, extending from the anterior limb bud to the posterior limb bud. That part of the line occupying regions which ordinarily do not possess mammary glands usually disappears; however, it happens in about 7 per cent. of human embryos that mammary glands develop from other parts of the mammary line besides the typical thoracic portion. Such glands which may appear in any position that was formerly occupied by the line are known as supernumerary glands. It is not uncommon to find an engorgement in the axilla or the inguinal region in the infant a few days after birth due to the engorgement of one or more supernumerary glands with new-born secretion.

Supernumerary glands also appear in the lower animals. In the rat Schickele,[15] Henneberg[1] and Myers[10] have observed supernumerary mammary glands.

Instead of an individual possessing an excess number of mammary glands the number may be reduced. This condition is more common in some lower animals, the rat for example, than the presence of supernumerary glands.

---

[15] Schickele, G.: Beiträge zur Morphologie und Entwickelung der normalen und überzähligen Milchdrüsen, Ztschr. f. Morphol. u. Anthropol. **1**: No. 3, 1899.

Effects of Inanition on the Developing Mammary Glands. — The effects of starvation on the development of the mammary glands have not been studied in many species of animals. It has been shown, however, in rats[16] that severe inanition retards the growth of the milk-ducts of the female rat during the first week of postnatal life, but apparently does not completely stop their growth. In rats held at birth weight for a longer time the ducts cease to grow and remain in a condition slightly more developed than at the time of birth. If after the first week the gross body weight of the animal is allowed to increase so as to correspond with that of a normal animal of one week, the milk ducts fail to develop to the same extent as those of a normal animal of corresponding body weight. This also holds true if the body weight of the underfed rat is allowed to equal that of a normal animal of two weeks. The growth of the milk ducts of male rats is retarded by inanition in a manner similar to that of the female. The nipple grows very little during inanition, being elevated above the surface only slightly in young rats starved severely for eight to ten weeks. The subcutaneous fat that appears very early in the neighborhood of the milk ducts soon becomes greatly decreased after the amount of food is reduced to a minimum. In all the retardation in the development of the mammary gland is roughly proportional to the retardation in body weight (at least within limits of normal variability).

Severe inanition for a short time at an early age thus temporarily stunts the mammary glands. When the animal is refed the glands respond slowly. When the body weight during refeeding reaches that of a normal at the age of puberty, the milk ducts are far behind those of the normal rat at corresponding body weight. That this stunting is not permanent is shown by the fact that the ducts ultimately attain the same stage of development as those of a normal rat, but at a much later period.

The question arises as to whether inanition at an early stage which temporarily stunts the development of the mammary glands affects the later functional stages of the glands. Further experimental work will aid in determining this point. A series of observations, extending over several years, on girls and young women who at some time had been subjected to a prolonged period of inanition would doubtless throw some light on the question as to whether temporary stunting later interferes with lactation.

---

[16] Myers, J. A.: Studies on the Mammary Gland. V. The Effects of Inanition on the Mammary Gland in Male and Female Albino Rats from Birth to Ten Weeks of Age, Am. J. Dis. Child. **17**:311 (May) 1919. (Abstr. published in Anat. Rec., May, 1919.)

Comparison of the Developing Mammary Glands in the Two Sexes. —It is frequently stated that the mammary glands of the two sexes show a parallel development until the time of pregnancy when the glands of the female undergo rapid development, while those of the male show retrogressive changes. This statement is undoubtedly correct in the case of the glands of the female, but cannot be relied on to describe the changes in the glands of the male.

It has been shown in the rat[17] that while the sex glands are in the indifferent embryonal stage there is no apparent difference between the anlages of the mammary glands in the two sexes. From this stage the glands of male and female animals present a parallel development until about the fifth week of postnatal life except that in this particular species the male fails to develop nipples. After the fifth week the milk ducts of the female present more branches than those of the male. The difference is not very great, however, until about the time of puberty, when the ducts of the female branch very profusely, while the ducts of the male show but little change. Observations were made on a few male rats between the ages of ten and sixteen weeks, and it was found that the milk ducts of the sixteen week rats were found somewhat better developed than in those of nine and ten weeks. Apparently considerable growth had taken place and new ducts had developed as the arborization of the ducts appeared more dense.

Kölliker[18] found that in humans from one to ten years after birth the milk ducts and end buds increase only a little, branching somewhat more richly in the female than in the male sex. Krause[19] also states that there is no essential difference in the glands of the two sexes at birth; the differences belong to later periods of life. Broman[19] states that in the human the glands of the male do not develop after birth. My observations on infants at the University of Minnesota hospitals have confirmed the findings of numerous investigators who have reported that the new-born secretion is present and has the same appearance in the glands of the two sexes. Attention should be called to the fact that Kölliker[18] found that in men of 20 years mammary gland vesicles were present which were not observed at about the time

---

17. Myers, J. A.: Studies on the Mammary Gland. III. A Comparison of the Developing Mammary Glands in Male and Female Albino Rats from the Late Fetal Stages to Ten Weeks of Age, Anat. Rec. **13**: 1917. (Abstr. published in Anat. Rec. **11**: 1917.)

18. Kölliker, Th.: Beiträge zur Kenntniss der Brustdrüse, Verhandl. d. phys. med. Gesellsch. zu Würzburg, N. F. **14**: 1879.

19. Krause, W.: Die Entwickelung der Haut und ihrer Nebenorgane, Hertwig's Entwickelungslehre der Wirbeltiere **2**: Parts 1 and 2, 1906.

19. Broman, Ivar: Normale und abnorme Entwicklung des Menschen, 1911.

of puberty. It was not until about the thirtieth year that Kölliker found retrogressive metamorphosis beginning in the mammary glands of men. Furthermore, Merkel[20] and Kerr[21] state that occasionally at the time of puberty some swelling and soreness are experienced in the mammary glands of men. Therefore it must be concluded that the mammary glands in the male may show some growth after puberty.[22]

---

[20] Merkel, Fr.: Handb. der topograph. Anat. **2**: 1899.

[21] Kerr, A. T.: The Anatomy of the Breast, Ref. Handb. Med. Sc. **2**: 445, 1916.

[22] Schultze, O.: Ueber die erste Anlage des Milchdrüsenapparates, Anat. Anz. **8**: 1892.

Reprinted from the American Journal of Diseases of Children
July, 1919, Vol. XVIII, pp. 4-13

American Medical Association
Five Hundred and Thirty-Five North Dearborn Street
CHICAGO

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MARCH 30

Reprinted from JOURNAL OF MORPHOLOGY,
Vol. 31, No. 1, June, 1918

187

THE MORPHOGENESIS OF THE THYREOID GLAND IN SQUALUS ACANTHIAS

E. H. NORRIS

From the Institute of Anatomy, University of Minnesota

TWENTY-THREE FIGURES

CONTENTS

1. Introduction 187
2. Material and methods 188
3. Prefollicular period 190
    a. Organogenesis 190
    b. Histogenesis 192
4. Follicular period 198
5. Blood supply 204
6. Discussion and conclusions 210
7. Summary 216
8. Literature cited 219

1. INTRODUCTION

The morphogenesis of the elasmobranch thyreoid presents many problems of more than intrinsic interest and import, because of their bearing upon the large number of researches regarding the development and structure of this organ throughout the vertebrate series. The thyreoid gland of this form shows with almost diagrammatic clearness, in its earlier stages, a number of features which are rendered relatively obscure in forms which are more complex or undergo a more rapid development. The gland further exhibits a number of characteristics, both during its early development and during the follicular period, which are of particular interest in the light of recent work on the human thyreoid gland (Norris, '16). Moreover, the organ presents a very fortunate field for a continued study of the relation of parenchymatous and vascular structures.

This study was undertaken in the Anatomical Laboratory of the University of Minnesota at the suggestion of Prof. R. E. Scammon, under whose supervision the work was conducted.

I wish to thank Dr. Scammon for his valuable aid and criticism and for the loan of material from his embryological collection.

2. MATERIAL AND METHODS

The present paper, based upon an extensive series of embryos of Squalus acanthias, gives an account of the development of the thyreoid from the time of its earliest appearance until the adult structure is attained.

The following table, in which the embryos are arranged in the order of their lengths, shows the specimens used in this work. A large part of the material is from the Harvard Embryological Collection (H.E.C.), while some is from the Embryological Collections of the Department of Anatomy of the University of Minnesota (M.E.C.), the personal collection of Dr. R. E. Scammon (S.C.), and the collection of the Department of Zoology of the University of Kansas (K.U.E.C.). The material marked M.E.C., as well as the older new-born specimens, were obtained by Dr. Scammon from the Harpswell Biological Laboratory. My thanks are due for the loan of material. An asterisk (*) following the collection number signifies that the cervical region of the embryo alone was sectioned. Otherwise the entire embryo was available in serial sections.

The ordinary reconstruction methods, both plastic (Born's waxplate method) and graphic, were utilized in the present study. In all cases where a determination of the follicular form or structure was attempted, special precautions were observed in making the reconstructions as accurate as possible.

The drawings for reconstruction were made with the camera lucida or with Edinger's projection apparatus on transparent paper. After the drawings were completed, those of successive sections were superimposed upon a tracing-table, and the corresponding structures in each section were then given a letter or number. The drawings were controlled throughout by careful microscopic observations, to determine the frequently complicated relations of neighboring follicles. By this method it was possible to determine with certainty the limits of any particular mass or follicle.

SERIAL NUMBER	COLLECTION NUMBER	LENGTH IN MILLIMETERS	SECTION THICKNESS IN MICRONS	CORRELATION OF MATERIAL[1] WITH	
				Normal-plate numbers	Balfour's stages
1	M. E. C. 685	2.0	7	7	D, E, F
2	M. E. C. 686	3.0	7	10	G
3	H. E. C. 1498	3.8	8	17	H
4	M. E. C. 679	4.0	7	16	
5	K. U. E. C. 449	5.0	10	20	
6	M. E. C. 614	5.0	7	20	
7	H. E. C. 1335	5.2	6	20	
8	S. C. 17	5.4	10	19	
9	K. U. E. C. 545	5.8	10	19	
10	H. E. C. 1497	5.8	8	19	I
11	H. E. C. 1821	6.0	10	19	
12	M. E. C. 612	6.0	7	21	
13	S. C. 29	6.3	10	21	
14	S. C. 15	7.5	10	22	
15	S. C. 24	7.5	10	22	
16	M. E. C. 615	7.5	7	22	
17	S. C. 12	7.5	10	22	
18	H. E. C. 1495	9.0	8	23	
19	S. C. 20	10.0	10	23	
20	S. C. 16	10.1	10	23	K
21	S. C. 50	11.5	20	24	
22	M. E. C. 645	11.6	7	24	
23	S. C. 18	13.3	10	25	L
24	S. C. 3	14.0	10	26	
25	M. E. C. 613	15.0	7	26	
26	S. C. 1	15.5	10	26	
27	S. C. 52	18.0	10	27	
28	S. C. 4	19.0	10	27	
29	M. E. C. 646	19.0	8	27	
30	S. C. 2	19.0	10	27	M
31	M. E. C. 647	19.8	8	27	
32	S. C. 5	20.5	10	27	
33	H. E. C. 1494	20.6	10	28	N
34	H. E. C. 1493	21.0	10	28	
35	S. C. 53	22.0	10	28	
36	M. E. C. 629	24.0	10	29	
37	S. C. 6	28.0	10	30	
38	H. E. C. 1357	28.0	10	30	O
39	S. C. 25	33.0	12	31	
40	M. E. C. 680	33.0	10	31	
41	S. C. 8	33.1	12	31	
42	S. C. 9	36.0	12	32	
43	S. C. 7	36.0	12	32	

SERIAL NUMBER	COLLECTION NUMBER	LENGTH IN MILLIMETERS	SECTION THICKNESS IN MICRONS	CORRELATION OF MATERIAL[1] WITH	
				Normal-plate numbers	Balfour's stages
44	S. C. 10	36.5	10	32	
45	S. C. 11	48.0	12		
46	S. C. 54	48.0	12		
47	H. E. C. 427	60.0	14		
48	S. C. 55	80.0	12		
49	H. E. C. 1882	95.0	12		
50	New-born 2*	160.0	10		
51	New-born 5*	160.0	10		
52	New-born 1*	170.0	10		
53	New-born 6*	200.0	10		
54	New-born 3*	210.0	10		
55	New-born 4*	ca.250.0	7		

[1] In correlating embryos of the present series with Normal-plate numbers and with Balfour's stages only the general development of the embryo has been considered and not the state of development of the thyreoid gland.

3. PREFOLLICULAR PERIOD

a. Organogenesis

The anlage of the thyreoid gland in Squalus acanthias makes its appearance in embryos of approximately 4 mm. in length as a solid epithelial bud from the floor of the pharynx. This bud, which is at first little more than a localized thickening of the entodermal lining of the pharynx, is placed just ventral to the point at which the oesophagus leads from the pharynx and in the region inferior and caudal to the ventral extremities of the first two pairs of gill pouches (figs. 1, 2, 17). A study of these figures will show the absence of any groove or pouch in the floor of the pharynx at the point where the thyreoid first appears. Not only is there an entire absence of a groove or pouch, but, as shown in figures 1 and 2, the anlage even protrudes somewhat into the cavity of the pharynx. The bud increases in size and, extending caudally, assumes a pedunculated form, being suspended from the floor of the pharynx by a rather extensive but very narrow neck. This condition obtains in embryos of 5.8 mm. (No. 9 of present series), where the gland mass and the neck by which it is suspended are nearly coextensive. By the time the embryo has reached 14 mm. in length the thyreoid is better described as keel-shaped (fig. 18). The gland mass and the suspending neck are no longer coextensive, for the former has grown caudally until it is nearly three times as long as the latter. By the time the embryo has attained a length of 19 mm. the gland has severed its connection with the pharynx and has the form of a column with rounded ends and whose cross-section is ovoid (fig. 19). But the gland does not long preserve its columnar form. By the time the embryo has reached a length of 24 mm. the gland is well advanced in its transition, being neither columnar, as was the condition at 19 mm., nor completely flattened, as in the succeeding stages. This transitional stage is well set forth in figure 20. At 28 mm. the gland has quite lost its columnar form of the earlier periods and has assumed the form of a flat, diamond-shaped plate whose surfaces are quite smooth and regular (fig. 21).

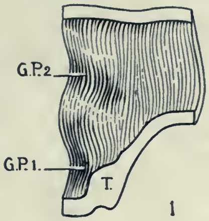


Fig. 1 Graphic reconstruction of the pharynx of an Acanthias embryo 3.8 mm. long (No. 3). *G.P.*1, and *G.P.*2, first and second gill pouches, respectively; *T.*, thyreoid. × 75.

Between 28 and 33 mm. the gland again changes in form, and only at this stage (33 mm.) can it be said of the organ for the first time that has in miniature the form of the adult thyreoid (fig. 22). For descriptive purposes the gland is readily divisible into two parts, an anterior, the corpus, and a posterior, the cauda. The corpus, as seen from above or below, is rhomboidal in outline, its transverse diagonal being a little greater than its anteroposterior; while the cauda, on the other hand, presents the form of a long straight bar which joins directly with the posterior angle of the corpus in front and terminates posteriorly in a pointed extremity. The body is nearly three times as thick as the tail, but the two parts are quite alike as regards regularity of outline and smoothness of surface. The ventral surface of the tail is grooved along nearly its whole extent by a closely applied blood-vessel.

In embryos between 33 mm. and 50 mm. in length (which latter approximately marks the end of the prefollicular period), the gland continues its growth and increases considerably in size. But notwithstanding the increasing mass of the organ, it constantly maintains through succeeding stages an outline which simulates that of the stage last described.

One further observation on the external form should be noted. Almost without exception, the glands, subsequent to the period in which they sever their connection with the pharynx, present a small mound-like projection from the dorsal aspect of the body (corpus). This little mound is quite regularly located in the central region of this surface.

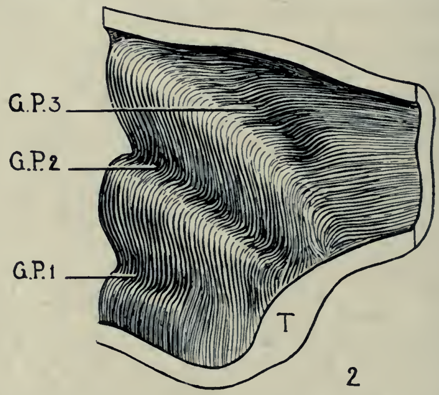


Fig. 2. Graphic reconstruction of the pharynx of an Acanthias embryo 5 mm. long (No. 6). *G.P.*1, *G.P.*2, and *G.P.*3, first, second, and third gill pouches, respectively; *T.*, thyreoid. × 75.

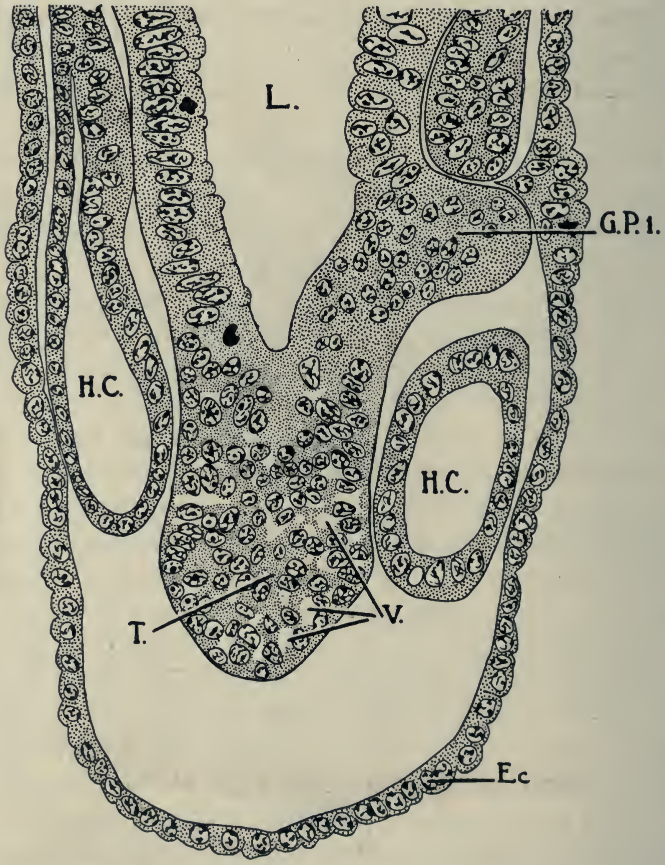
b. Histogenesis

The modifications in the external form of the gland are accompanied by, and to some extent at least, dependant upon a number of internal changes.

The anlage of the thyreoid gland when studied in cross-section appears as a solid diverticulum or bud from the floor of the pharynx. The cells which make up this bud are quite like those of the entoderm from which it is derived. The cell outlines are not apparent. The thyreoid nuclei, which are all very similar, contain one, two or three large masses of chromatin, which are round or oval in shape and smooth in outline, except for one or two small chromatin threads which may extend outward from each mass. These chromatin masses are generally closely applied to the nuclear membrane. The remainder of the nucleus is filled with a clear nuclear sap through which run a few delicate and faintly staining fibrils. This type of nucleus, which has been described and figured by McGill ('10), Neal ('14), and Scammon ('15), is characteristic of the young Acanthias embryo, being found also in the cells of the mesenchyma, mesothelium, medullary canal, in the hepatic anlage, and elsewhere. In the earliest stages, all of the nuclei are placed with their long axes perpendicular to the surface of the thyreoid bud.

This condition is present only in the early anlage while the gland mass is diminutive. As soon as the gland increases in size (embryo of 5 mm.), changes are noticeable both in the structure and arrangement of the nuclei. The chromatin masses above mentioned become more irregular and there are given off from them a number of chromatin strands which eventually form a coarse network. The chromatin masses may also be somewhat broken up, being separated from the nuclear wall so as to present a granular appearance. But the change in form of the nuclei is perhaps even more striking than their altered structure. In place of the elongated ovoidal nuclei of the early anlage, which were in all respects like the nuclei typical of the general entoderm, the gland at this stage presents nuclei which appear in the cross-sections to be nearly spheroidal. These nuclei have diameters which are approximately the same as the short diameter of the nuclei found in the general entoderm. Those which are most ovoidal lie in the peripheral part of the gland and are placed with their long axes perpendicular to the surface. The nuclei of the central part of the gland, on the other hand, are very irregularly disposed. At this same stage (embryo of 5 mm.) a large number of vacuole-like spaces can be seen which are quite generally distributed throughout the gland mass. Whether these are intra- or intercellular spaces cannot be said, for the cell boundaries are not visible. The margins of these spaces are extremely irregular and indistinct, blending almost imperceptibly with the faintly staining cytoplasm. These changes are shown in figure 3.

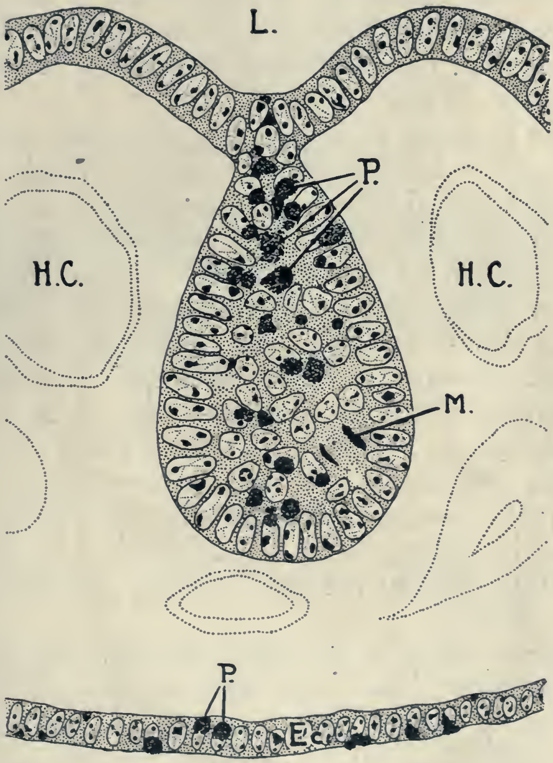
In embryos of 5.8 mm. the structure of the gland is again changed. As pointed out in the preceding section, the thyreoid of



3

Fig. 3 Drawing of a part of a cross-section of an Acanthias embryo 5 mm. long (No. 6). *Ec.*, ectoderm; *G.P.*1, first gill pouch; *H.C.*, head cavity; *L.*, lumen of pharynx; *T.*, thyreoid; *V.*, vacuole-like spaces. × 300.

this stage has the form of a pedunculated bud suspended from the floor of the pharynx by a very narrow neck. Figure 4 is a cross-section of the gland at this stage and shows the pedunculated form as well as the structure of the bud. No cell boundaries are apparent in this region, either in the entoderm lining the pharynx or in the glandular epithelium. The nuclei are quite characteristic in form, arrangement, and structure. As regards form and arrangement, two general types of nuclei may be noted; the first, those which are elongately ovoidal and which are found in a cir-



4

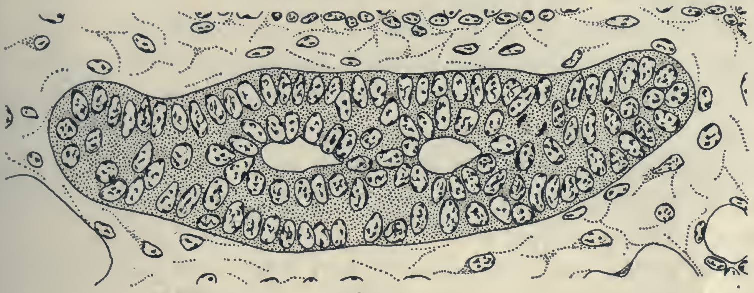
Fig. 4 Drawing of a cross-section through the thyreoid gland and adjacent structures of an Acanthias embryo 5.8 mm. long (No. 9). *Ec.*, ectoderm; *H.C.*, head cavities (outlined in stipple); *L.*, lumen of pharynx; *M.*, mitotic figure; *P.*, pigment granules. × 500.

cumferentially disposed row around the periphery of the thyreoid bud, as well as in the cells lining the pharynx. The second type, those which are spheroidal or at most slightly ovoidal, are found both in the center of the thyreoid bud and in its suspending stalk. Structurally, these two types of nuclei are very similar. They possess a distinct nuclear membrane and regularly contain two large, smooth, chromatin masses which do not lie in apposition with the nuclear wall. These chromatin blocks are suspended in a clear nuclear sap through which run a few faintly staining linin fibrils. Certain vacuole-like spaces, similar to those described in the gland of a 5-mm. embryo, are also present in this stage and may be noted in the succeeding members of the series up to approximately 20 mm.

As shown in figure 4, the gland at this stage (embryo of 5.8 mm.) contains a large amount of pigment which is scattered throughout the gland mass, but is accumulated in largest quantities in the neck of the bud. This pigment is made up of highly refractive, yellowish-brown granules which are closely packed together in masses or blocks of variable size. Whether this pigment is intra- or intercellular cannot be determined with definiteness. Certain of the blocks seem to be superimposed upon underlying nuclei, while others appear to be lodged in the cellular interspaces. A similar pigment is found in the ectoderm of this same region (fig. 4). This pigmentation of the gland occurs in all the specimens from 5.8 mm. up to about 19 mm., at which time the gland severs its connection with the pharynx. Thereafter the pigment decreases rapidly, a little being present even up to 24 mm.

Histologic evidence of the rapid growth of the gland during these early stages is seen in the large numbers of mitotic figures found in nearly every section. In a single longitudinal section through the gland of a 21 mm. embryo, ten cells in karyokinesis were counted.

At 19 mm. there appear within the gland a number of completely closed cavities. These cavities are at first only tiny clefts, appearing very much as though the cells around them had only pulled apart a little in their formation. These small clefts soon increase in size and are thus brought more closely into relation with one another. Nothing more can be said by way of generalization regarding their size, shape, or arrangement, great differences being found in these respects both in cavities of the same gland as well as in glands in different stages of development. These intraglandular cavities are not at all like the vacuole-like spaces described above. In every case these cavities, unlike the vacuole-like spaces, are outlined by a very distinct and sharp margin, and in no case is there any visible content within the cavity. It is important to note that these spaces are quite independent of any external conditions, and in no case in these early stages do they open to the outside of the gland. Furthermore, as shown in figures 5, 19, 20, 21, 22, they develop independently of one another as isolated spaces and only secondarily may become confluent. At a much later stage (28 mm.) these cavities, for the first time, open to the outside and are invaded by blood-vessels. These cavities develop only in the body of the gland and have never been observed in the region of the tail (cauda).



5

Fig. 5 Drawing of a cross-section through the thyreoid gland of an Acanthias embryo 28 mm. long (No. 37). × 450.

Careful examination of sections of the gland in this period of its development shows that this process of cavity formation is accompanied or paralleled by—probably even preceded by—four other processes, in all of which the cells actively participate. The first of these is the beginning differentiation of cell boundaries, which originally are very faint and indistinct, but which are well formed and easily seen some time before the cavities have opened to the outside.

The second process consists of a change in form of the nuclei. The centrally located spheroidal nuclei become ovoidal like those occurring in the periphery.

The third process is very closely allied with the second and has to do with the rearrangement of the nuclei. The peripherally disposed nuclei maintain the position they have constantly held, while those of the central part of the gland which have become ovoidal, instead of remaining irregularly scattered, now assume a definite relation to the developing cavities. As a result of their rearrangement, their long axes are placed perpendicular to the margins of the cavities. By this process the vast majority of the nuclei in the thyreoid establish a definite relation to some glandular surface, whether external or internal.

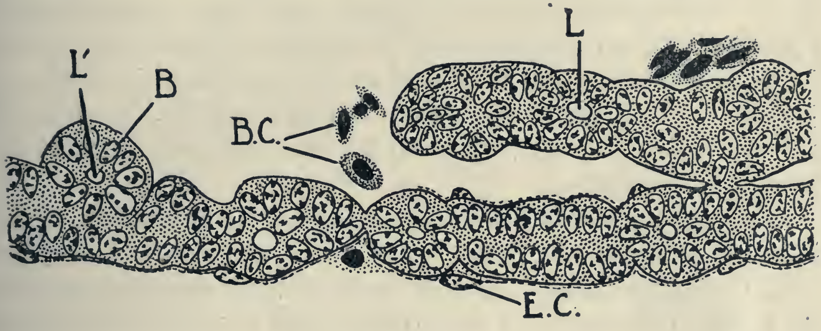
The fourth process, that of cell proliferation, is, as already mentioned, remarkably rapid during this period.

As a result of these processes, the gland is changed from the form of a solid epithelial column into a number of epithelial plates. These plates, which are two cells in thickness, possess surfaces which are quite smooth and which are very intimately related to the blood vascular system. It is from these plates that the primary follicles develop.

4. FOLLICULAR PERIOD

A thyreoid follicle may be defined as a completely closed sac whose wall is usually made up of only a single layer of epithelial cells. As pointed out in a previous paper on the morphogenesis of the follicles in the human thyroid gland (Norris, '16), this definition includes all the features of the follicle which may be regarded as absolute and constant. The size and shape of the follicle may vary, and great differences are found in these respects in follicles of the same gland as well as in follicles of embryos at different stages. Typically the thyreoid follicle may be considered spherical or spheroidal in shape; but, as will appear later, this type is subject to considerable variation. The term primary follicle will be used to include those follicles developing independently of pre-existing follicles. The follicles derived from pre-existing follicles, by budding or otherwise, are termed secondary follicles.

In the present series, the first primary thyreoid follicles appear in an embryo 60 mm. long (No. 47). In this specimen the thyreoid gland has essentially the same structure as has that of the last stage described in the prefollicular period, i.e., it is made up chiefly of longitudinally placed epithelial plates or bands, which are only two cells in thickness. But in this stage, the plates, which have in previous stages been characterized by comparatively smooth surfaces, now present surfaces which are more or less roughened by the appearance of scattered hillocks or mounds (figs. 6, 23). They are placed very irregularly with respect to one another, and may appear for the first time in any part of a plate, at its periphery or in a more central region.



6

Fig. 6 Small portion of a cross-section of the thyreoid gland in an Acanthias embryo 160 mm. long (No. 50), magnified to show cell structure and the relation of the developing primary follicles to the epithelial plates. Note the appearance of lumina (*L*) in buds (*B*) from the surface of the plate as well as in swellings along its course (*L'*). *B.C.*, blood corpuscle; *E.C.*, endothelial cell. × 450.

Cross-sections (fig. 6) show that these hillocks are the immediate anlagen of the early thyreoid follicles. Further, the hillocks are apparently produced by the concurrence of four different processes in the epithelium. The first process is that of cell rearrangement, the second that of cell proliferation, the third that of absolute cell growth, and the fourth that of lumen formation. These processes, although described separately, may occur simultaneously.

The first departure from the two-celled plate arrangement, in the process of follicle formation, is found in a rearrangement of the cells of the plate (fig. 6). The cell outlines can be made out only with difficulty in most cases. But in those places where they can be seen, they bound cells which are more or less columnar in form. The nuclei are ovoidal or elliptical in outline and are placed with their long axes perpendicular to the surface of the plate. Here and there along the course of the plate (fig. 6) some of the nuclei have shifted their axes and have changed their relative positions. Certain of the nuclei have rotated through an arc of 90 degrees so that their long axes, in their final position, are at right angles to their original position in the plate. As a result of this shifting process, little circlets (really spheres) of nuclei are formed in the plate.

This shifting of the nuclei is but the visible expression of the changing position of the cell. For while it is impossible to observe the cell boundaries in most cases, it is hardly probable that the nuclei shift their axes independently of the cytoplasm; more over, the few faint cell-boundaries which may be made out show the same changes in position as do the nuclei. Further, it is usually found that at the point from which a nucleus has shifted toward the center of the plate a slight depression appears on the surface of the plate, indicating that the cytoplasm has shared equally with the nucleus in this movement. From these observations it may be concluded that the first process manifested in follicle formation is the shifting of the axes of certain cells of the epithelial plate through an arc of 90 degrees.

This process results in the transformation of the smooth surfaces of the bands (fenestrated plates) into surfaces which are somewhat roughened. Apparently the irregularities are not due, at first, to swellings on the plates, but rather to the slight indentations produced by the shifting of certain cells toward the center of the plate as above described. In cross-sections (figs. 6, 13, 14) such a plate appears as a sort of beaded chain, with alternate swellings and constrictions. But, as noted above, the initial swellings due to this process are only apparent and actually are not greater in thickness than is the plate in other parts of its extent where indentations have not yet occurred.

The extraordinary cellular activity of the epithelium at this stage is clearly manifested by the large number of mitotic figures. The localization of these mitoses is even more significant than is their number. The nuclear figures are usually found only in those places in the epithelial plates where actual thickenings on the plates are being formed. Therefore the little mounds which appear on the plates, as the immediate anlagen of the early follicles, may be formed not only by the rearrangement of the already existing cells of the epithelial plates, but also by the formation of new cells as well. Consequently, it can easily be seen how the apparent swellings on the plates, produced by the rearrangement of the existing cells, may be transformed into actual swellings by the absolute increase in number of the cells found in a localized area. These swellings become roughly spheroidal in form.

The third process referred to above is the absolute increase in size of the cells. While the cells are shifting their axes nd proliferating, they are also increasing in size. This progressive increase in the height of the cells corresponds in general to the progressive stages in the differentiation of the two-celled plate into newly formed follicles. So that the thyreoid gland of an embryo 60 mm. long presents, in different regions, epithelial cells varying greatly in height. The lowest cells are found, at the beginning of the process, in the two-celled plate; the highest being found at the other extreme, in the completely formed follicle.

Three of the four processes above mentioned as apparently involved in the evolution of the follicle from the epithelial plate have now been reviewed in detail. The formation of the lumen remains to be considered. Just preceding the appearance of the lumen, the spherule (in which it is about to develop) appears in cross-section as a circlet of columnar cells, whose nuclei are peripherally placed. This arrangement results in the formation of a striking picture. The nuclei are regularly placed at the periphery of the circle and form a dark band, which surrounds a clear, central cytoplasmic portion. The magnitude of this cytoplasmic area and the sharp contrast between the two portions (in the stained preparations) are usually prominent features. The lumen makes its appearance in the center of this cytoplasmic area as a tiny spherical space outlined by a definite and regular margin. It is as though the cells had but drawn apart a little, so that their central ends, instead of remaining in contact with one another, are separated by an interval. It is important to note that no tubular stage is found in the process of lumen formation, but, as was pointed out in the case of the human thyreoid gland (Norris, '16), the lumina appear as absolutely independent spaces.

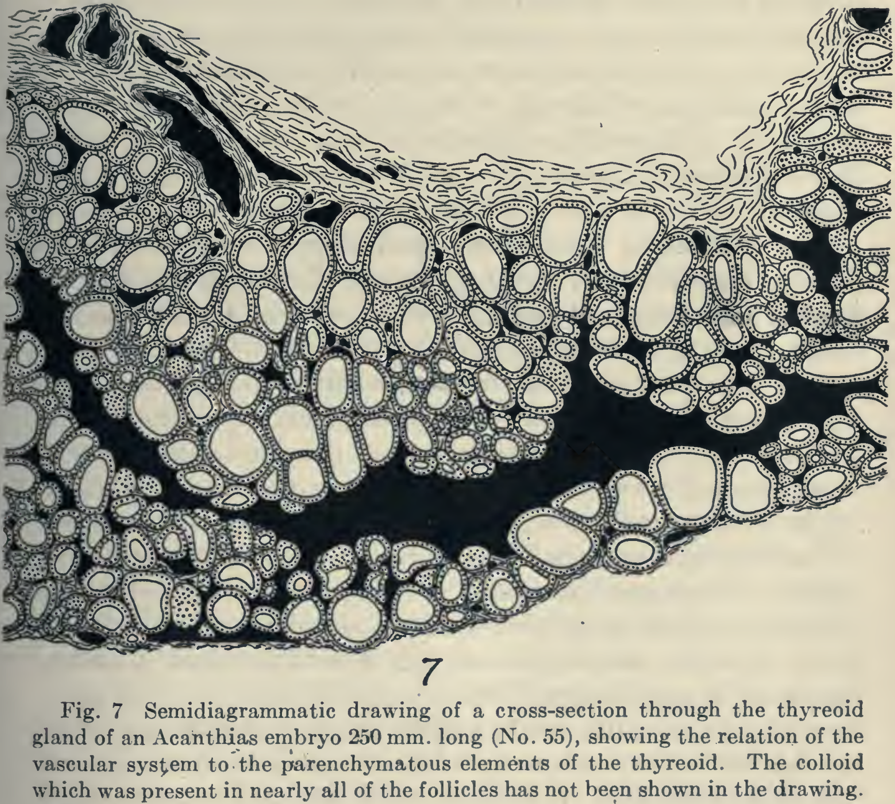
When the lumina first appear they have no apparent content; but undoubtedly they contain some substance which is not stained with the ordinary method and which increases in amount with the size of the follicular cavity. Certain of the larger lumina (not all of them), which are perhaps older, are found to contain a hazy, granular substance. Typical colloid does not appear until later, in the 160-mm. stage (No. 50).

In the foregoing account, the cell masses in which the lumina develop have been described as spherules whose cross-section is circular in outline. While this is true for typical follicles, some variation, within comparatively narrow limits, is found. Ovoidal or somewhat irregular follicles occur, but these are not more numerous than would be expected in a rapidly growing tissue.

These stages, which have just been described, may be summarized very briefly. The comparatively smooth epithelial plates of the prefollicular period have been transformed into plates with rough surfaces. The roughenings on the plates are the early indication of the follicles about to be formed. With the progressively increasing number of follicles the plates are transformed into irregular bands, which in turn give rise to groups of solid or hollow masses of cells.

Although the first well-formed primary follicles are found in embryos about 60 mm. long, the epithelial plates of the prefollicular period are only very gradually transformed and replaced by follicles derived from them. Primary follicles continue to be formed until the embryo attains a length of approximately 250 mm. Apparently, by this time, the epithelial plates have been completely used up in the formation of follicles.

At 250 mm. the thyreoid is made up of follicles which are variable both in size and form. Apparently the follicles tend to assume a spheroidal form, but through crowding and mutual pressure of the adjacent elements, they are forced to assume ovoidal or irregularly angular shapes (fig. 7). The follicle wall consists of a single layer of low columnar epithelial cells which show evidences of secretory activity. Colloid, which begins to appear at about 160 mm. is abundantly present in the 250 mm. stage.



7

Fig. 7 Semidiagrammatic drawing of a cross-section through the thyreoid gland of an Acanthias embryo 250 mm. long (No. 55), showing the relation of the vascular system to the parenchymatous elements of the thyreoid. The colloid which was present in nearly all of the follicles has not been shown in the drawing. × 40.

Up to and including the 250 mm. stage, no evidences of the formation of secondary follicles have been observed. The material available from stages later than those included in the table of embryos studied was not suitable for histologic study. However, the thyreoids from three adult specimens were obtained and, although the glands were poorly preserved, certain observations were possible. A number of irregular follicles with bud-like processes are present which suggest the formation of secondary follicles.

5. BLOOD SUPPLY

From the time of its first appearance the thyreoid gland in Squalus acanthias is associated more or less intimately with the vascular system. In the early embryos, in which the gland is just recognizable, the ventral aorta bifurcates immediately caudal to the gland, so that its two most anterior branches (first arches) pass dorsally on either side of the thyreoid. These thin-walled vessels lie in immediate apposition with the thyreoid anlage (fig. 8), and although they give off no branches to the gland, they probably suffice to nourish all the tissues of this region.

Soon after these vessels have associated themselves with the thyreoid, mesenchyma begins to invade the region and to grow around both the vessels and the gland, so that by the time the embryo has reached a length of 5.8 mm. (No. 645) the mesenchyma, through its rapid growth, has formed a dense tissue which separates the blood-vessels from the gland for a considerable distance (fig. 9). This relation between the gland and the vascular system is maintained until the time at which the gland is cut off from the pharynx (ca. 19 mm.). At this stage another set of vessels begin to invade the region in which the thyreoid is placed. These are thin-walled venules growing forward from a trunk which buds from the common cardinal vein just before it opens into the sinus venosus. This venous trunk (external jugular or linguofacial vein) buds from the common cardinal in embryos of about 14 mm. in length. It grows downward along the side of the pericardium and comes to lie ventral to the heart and to the thyreoid. It breaks up into a wide-meshed network of sinusoidal venules around the latter (figs. 4, 15). This condition is found in embryos of about 19 mm. in length.

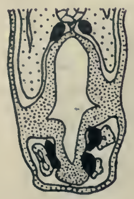
In an embryo of 20.6 mm. (No. 33) this venous plexus establishes the first apparent communication with the arterial system, through anastomoses which it forms with small twigs derived from the mandibular branch of the first aortic arch. The association of the thyreoid with the arterial system is never very extensive and the arterial blood which the gland receives is small.

The subsequent changes in the vascular system in its relation to the thyreoid gland are found to be: first, a more intimate association of vascular and parenchymatous elements from stage to stage; second, a marked and progressive increase in the area of the vascular bed locally; third, a very evident tendency of the venules around the thyroid to fuse and by their confluence to form the large 'thyroid sinus' (Ferguson, '11). These changes are clearly portrayed in the series of figures 10 to 15.

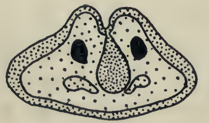
Apparently the blood-vessels grow around the thyreoid gland and spread out in the spaces between its plates without exerting any appreciable mechanical influence on it. The gland is, as it were, passively enveloped by the coalescence of the vessels around it. Although this is true in the early stages, during the latter part of the follicular period the follicles increase in size so as to considerably decrease the relative expanse of the thyreoid sinus. By their growth the lumen of the thyreoid sinus is encroached upon, one main drainage channel of considerable size being left, and a large number of capillary sinusoids (Minot, '00) are formed (fig. 7).

The vessels appear to have no causal relationship to the formation of the follicles, as the follicles do not begin to appear until after the sinus is well formed. Moreover, as noted above, the follicles of the Selachian thyreoid develop in the same manner as those of the man, and in this latter form there is no such relation between the glandular elements and the blood-vessels.

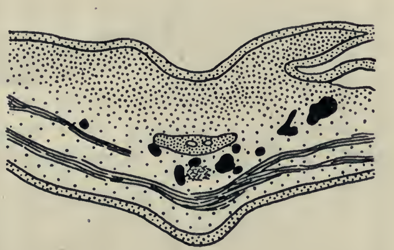
The endothelium lining the sinusoids forms a more or less complete investment for the epithelial portions of the gland. In most places the endothelium is present, but in some parts the epithelium appears to be in immediate contact with the blood stream.



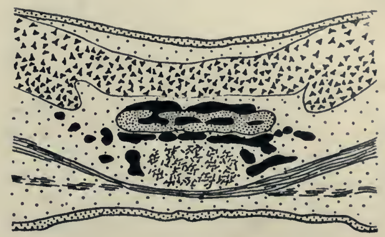
8



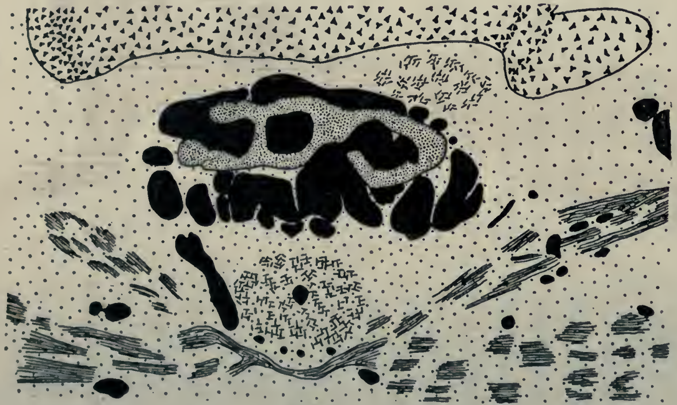
9



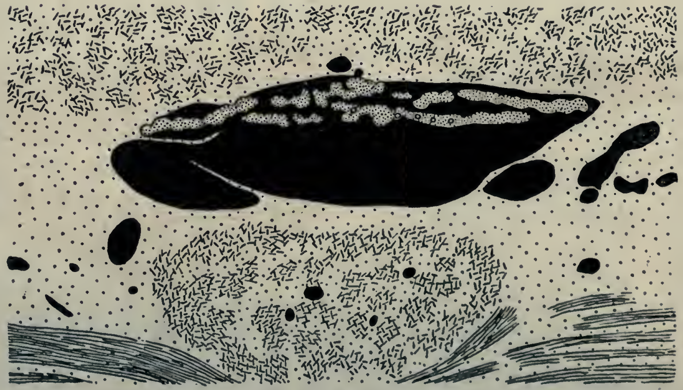
10



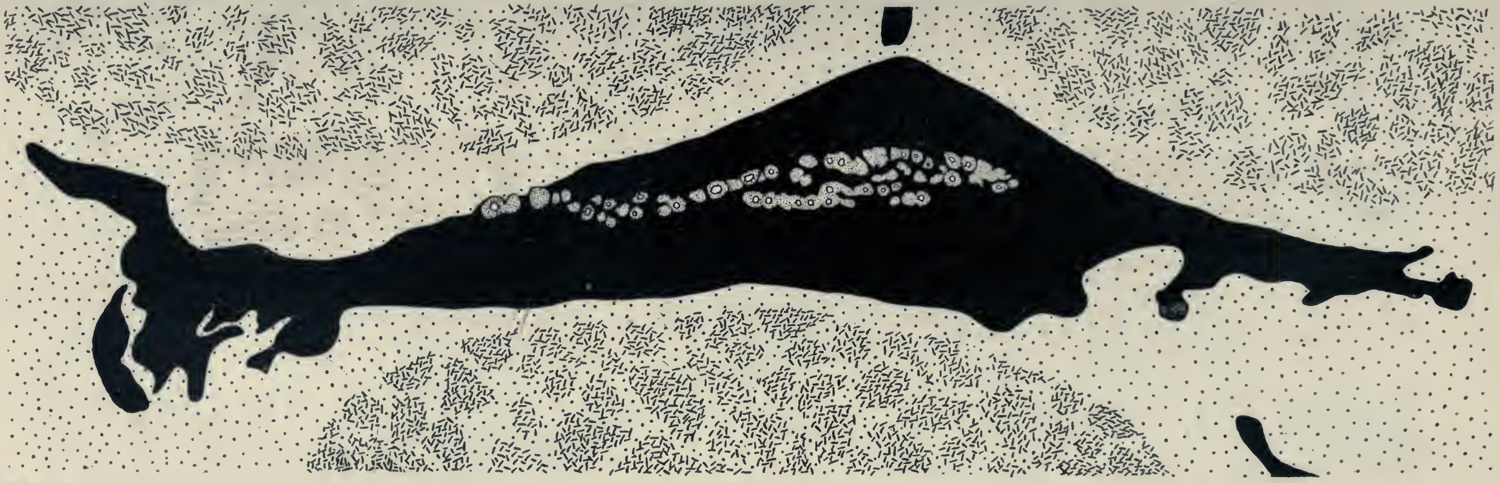
11



12



13



14

Figs. 8 to 14 A group of semidiagrammatic drawings (camera lucida outlines) showing the vascular relations of the thyreoid during the successive stages of its morphogenesis. The vascular elements are shown in solid black and the epithelial portions in a fine close stipple. Mesenchyme is shown in loose stipple; muscle in lines; cartilage in coarse stipple. × 45.

Fig. 8 Cross-section through the pharyngeal region of an Acanthias embryo 5.2 mm. long (No. 7). The thyreoid bud is shown attached to the ventral wall of the pharynx. The first aortic arch of either side lie in contact with the lateral surfaces of the bud. The anterior head cavities lie lateral to the aortic arches. The pharynx, the second gill clefts, the dorsal aortae, and the lower half of the notochord are also indicated.

Fig. 9 Cross-section through the mandibular process of an Acanthias embryo 14 mm. long (No. 24). The thyreoid gland is shown as a pedunculated bud attached to the pharyngeal floor. The aortic arches are separated from the thyreoid bud by a considerable mass of mesenchyma. Two anterior head cavities are located ventrolaterally to the thyreoid.

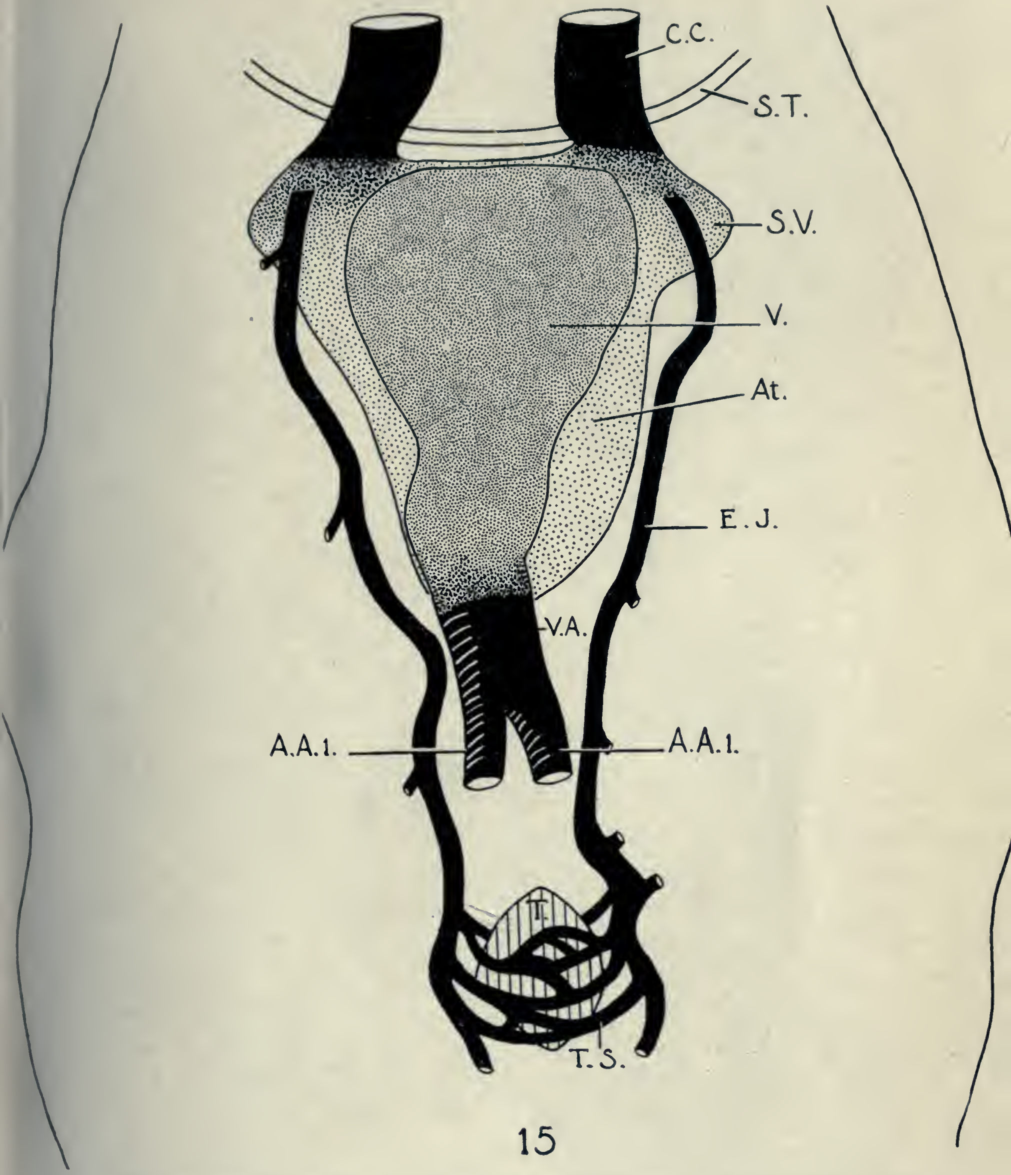
Fig. 10 Cross- section through the ventral wall of the pharynx of an Acanthias embryo 28 mm. long (No. 38). Thyreoid gland is shown near the center of the figure as a flattened structure having two closed cavities within. A considerable amount of mesenchyma is present in which blood-vessels, muscles, and cartilage are being developed. The vessels are a part of the thyreoid plexus of venules. The most ventral circular muscle is the constrictor pharyngis. The longitudinal bundle just ventral to the thyreoid is the coraco-mandibularis. The dense mesenchyma dorsal to the thyreoid is the anlage of the basihyal cartilage. The most dorsal and ventral structures are entoderm and ectoderm, respectively.

Fig. 11 Cross-section of the ventral wall of the pharynx of an Acanthias embryo 33 mm. long (No. 40). Thyreoid gland is shown near the center of the figure as a flattened structure, having four cavities, all of which are invaded by blood-vessels. Blood-vessels have increased in size and number around the gland and have associated themselves more closely with its surfaces. The surfaces of the epithelial plates of the thyreoid are quite smooth. The ectoderm, constrictor pharyngis, and coracomandibularis muscles, the basihyal cartilage, and the entoderm have positions similar to those of the same structures in figure 10.

Fig. 12 Cross-section of a part of the ventral wall of the pharynx of an Acanthias embryo 48 mm. long (No. 45). Thyreoid gland is shown near the center of the figure as a structure made up of anastomosing epithelial plates which are quite regular in thickness and which have smooth surfaces. Blood-vessels have increased in size and number around the gland and have intimately associated themselves with its surfaces. The other structures shown are located similarly to the same structures in figure 11, with one exception. The left coracohyoid muscle is located between the thyreoid gland below and the basihyal cartilage above.

Fig. 13 Cross-section of a part of the ventral wall of the pharynx of an Acanthias embryo 95 mm. long (No. 49.). Thyreoid gland is shown near the center of the figure as a structure which is made up of epithelial plates which have rough surfaces. Follicles are being formed in the plates and a few follicular lumina may be seen. The blood-vessels have fused around the gland to form the thyreoid sinus within which the gland is suspended. Note the two valve leaflets at the left end of the sinus. The other structures shown are located similarly to the same structures in figure 11.

Fig. 14 Cross-section of a part of the ventral wall of the pharynx of a newborn specimen of Squalus acanthias 170 mm. long (No. 52). The thyreoid gland is shown near the center of the figure as a structure which is made up of follicles and a few plates in which follicles are being formed. The magnitude of the thyreoid sinus and the fact that the thyreoid is suspended within it from only two points are striking features. Note the valve leaflet at the left end of the sinus. The other structures shown are located similarly to the same structures in figure 11.



15

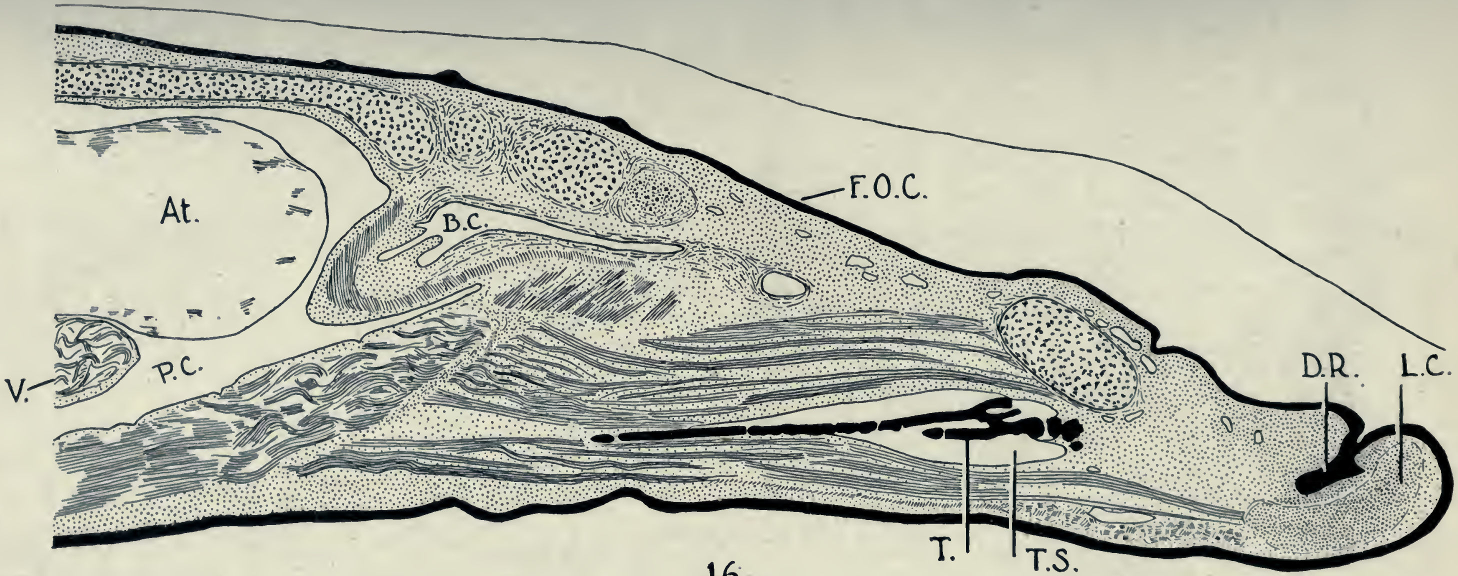
Fig. 15 Semidiagrammatic graphic reconstruction of the thyreoid gland and related vascular elements of an Acanthias embryo 28 mm. long (No. 38), seen from the ventral aspect. *A.A.*1, first aortic arch; *At.*, atrium, *C.C.*, common cardinal vein; *E.J.*, external jugular; *S.T.*, septum transversum; *S.V.*, sinus venosus; *T.*, thyreoid gland; *T.S.*, thyreoid sinusoids; *V.*, ventricle; *V.A.*, ventral aorta. × 8.

6. DISCUSSION AND CONCLUSIONS

The form of the thyreoid anlage in Squalus acanthias is of interest. As pointed out in the section on organogenesis, the anlage has the form of a localized, bud-like thickening of the pharyngeal epithelium (figs. 1, 2, 17). There is no thyreoid pouch or 'thyreoglossal duct' (His, '91) developed in any member of the present series, as has been described by W. Müller ('71) in an Acanthias embryo and by other observers in other forms. This may be a developmental peculiarity or, what seems more probable, it may be that the relative growth changes in the cervical region of the Acanthias embryo are less marked than they are in the same region of various other vertebrates, so that instead of being forced to change its relative position and to draw out a long connecting stalk, the gland remains in close relation to the pharyngeal floor until after its separation. The small mound-like projection from the dorsal aspect of the body of the gland, found in specimens immediately following the separation stage, probably marks the point of glandular detachment and may represent the thyreoglossal duct of other forms.

The fact that the relative position of the gland is changed very little is clearly shown in figure 16 of the present paper and also in a figure (fig. 3) published by Camp ('17). These figures indicate that the principal topographical change is brought about through the outgrowth of the long caudal portion of the gland, and that, although there is a small amount of growth forward from the anterior end of the body, there is almost no shifting of the point of detachment from the level at which the gland was originally set free. In other words, this point of detachment seems to remain through successive stages at a point about midway between the first and second gill clefts.

Goodey ('10) studied the relations of the adult thyreoid gland in three species of Selachians (Chlamdoselachus angineus, one specimen; Scyllium catulus, three specimens; Scyllium canicula, about thirty specimens) which are closely allied biologically to Squalus acanthias. In some of these he found varying degrees of connection between the thyreoid gland and the pharynx. In



16

Fig. 16 Midsaggittal section through the lower jaw and the structures located in the anterior and ventral portions of the body of an Acanthias embryo 60 mm. long (No. 47), showing the topography of the thyreoid gland. *At.*, atrium; *B.C.*, bulbus cordis; *B-h.C.*, basihyal cartilage; *D.R.*, dental ridge; *F.O.C.*, floor of oral cavity; *P.C.*, pericardial cavity; *T.*, thyreoid gland; *T.S.*, thyreoid sinus; *V.*, ventricle. × 25.

one case he found a tube-like structure extending from the pharynx through a foramen in the basihyal cartilage to the anterior end of the thyreoid, and in three other cases he found typical thyreoid tissue within the foramen of the basihyal cartilage. He concludes that this foramen "must have been occupied by the original entodermic evagination." These results of Goodey receive no support from the present investigation, for the thyreoid gland in Squalus acanthias has completely severed its connection with the pharynx some time before the basihyal cartilage is laid down. However, certain possibilities suggest themselves in attempting to harmonize these apparently conflicting observations. It may be that the thyreoid of the forms described by Goodey actually undergoes a somewhat different developmental history from that described in the present paper, or it may be that the cases in which he found adventitious thyreoid tissue are exceptional and represent cases of delayed separation of the gland.

The transformation of the gland from its columnar form (figs. 4, 19) to a plate-like structure, flattened dorsoventrally (figs. 5, 10, 11, 20, 21, 22, 23), is apparently due to pressure exerted by the development of surrounding elements, the chief of which are the basihyal cartilage above and the ventral musculature below.

What may be the significance of the vacuole-like spaces which appear within the early gland (fig. 3) cannot be said. Also an explanation of the meaning of the difference in form of the nuclei of the central and peripheral portions of the gland in these early stages (figs. 3, 4) is wanting. Balfour ('85), in describing the structure of the thyreoid of a Scyllium embryo which belongs in his stage L, points out a similar arrangement of the cells, but offers no suggestion of its meaning.

The large amount of granular pigment found in the parenchyma of the gland is a striking feature (fig. 4). This pigment increases in amount up to the time of separation of the gland from the pharynx, and thereafter rapidly disappears. It is massed in largest quantity at the point at which separation is to take place. Similar pigment is to be seen in the ectoderm ventral to the thyreoid region, and was also described by Balfour ('85) in the thyreoid of Scyllium embryos, and figured by Baumgartner ('15) in the hypophysis of Squalus acanthias. The pigment does not seem to be associated with nuclear or cellular degeneration, although in some cases the pigment blocks are superimposed on, and nearly obscure certain nuclei. The fact that this material is found in the ectoderm in the region near which the oral plate has opened, and in the thyreoid, chiefly in the neck region of the gland, suggests that it may be associated with any division or separation of epithelia.

After the gland has established its independence from the pharynx, its structure is again altered, as described above, by the development of completely closed cavities within the thicker parts of the gland mass. These cavities, although not previously described in the Selachian thyreoid, apparently correspond to the lumen which W. Müller ('71) mentions as present in the thyreoid of one of his Acanthias embryos, and to the cavities which Scammon ('11) observed in three specimens in the Normal-plate series (No. 29, 31, 32).

Since the gland has been regularly found to be solid up to the time of its separation from the pharynx, and since no thyreoid pouch or thyreoglossal duct has been observed, it is evident that these cavities develop quite independently of any earlier pouch, duct, or cavity. Further evidence in favor of this conclusion may be drawn from the fact that a number of these closed cavities or spaces of various sizes have been found in different stages of development not only in many of the glands, but also in different parts of the gland in the same specimen.

The cavities, which I first observed in the human thyreoid (Norris, '18) have been subsequently found in the thyreoid of Acanthias embryos (Norris, '17). In the human embryos, the cavities appear at a stage in the glandular development which exactly corresponds to the stage of their genesis in the fish thyreoid. Moreover, the rôle they play in the two forms is apparently identical. Born ('83), in describing the thyreoid glands of pig embryos 7 mm. long, mentions the presence of lumina in the lateral end of the gland mass. He does not include any description of these lumina, but they probably correspond to the cavities described in this paper. Since finding these intraglandular spaces in the thyreoids of human embryos and in those of Squalus acanthias embryos, I have made a brief study of a number of intermediate forms (pig, sheep, dog, pigeon) and have found similar cavities present in the thyreoid of each form investigated. It is hoped that these observations may be extended and presented in a subsequent publication. The existence of this same feature of morphogenesis in the thyreoid glands of two animal groups as far removed from one another as fish and man, as well as in the glands of a number of intermediate forms, will probably justify the conclusion that this is a fundamental feature of thyreoid development.

Any attempt to interpret these early intraglandular spaces either on the basis of their immediate or general biologic significance, although interesting, can be at best only speculative. It might be thought that the immediate purpose and significance of these cavities is to be found in the formation of the two-celled plates from which the follicles are later to be derived. On the other hand, if the vertebrate thyreoid is the phylogenetic representative of a true externally secreting gland (Patten, '17), it might be suggested that these cavities appear in response to a tendency to reproduce the ancient lumen or duct of the ancestral gland. This theory is attractive, inasmuch as it permits of harmonizing the known facts, both anatomical and physiological, regarding the endocrine function of the thyreoid. From this point of view the hypothesis and conclusion of Bensley ('16), "that the thyroid cell represents a true reversal of polarity," would be quite unnecessary. On the other hand, this phylogenetic theory would offer a plausible explanation of the present structure of the gland and its endocrine function without making it essential to hypothesize a reversal of cellular polarity.

It might be objected that the fact that these intraglandular spaces have no relation to the lumen of the thyreoglossal duct would appear to be a serious obstacle to the theory just advanced. But such a contention is not as formidable as it might seem at first sight, when it is considered that in the form at present under discussion no thyreoglossal duct is ever formed, and, moreover, even in those forms in which such a structure is sometimes developed, there are no specific cases described in which the lumen of the thyreoglossal duct is continuous with a cavity in the gland-mass proper. In other words, since the lumen of the duct does not extend into the body of the gland, any cavities found there must be morphologically independent of it, although they may, as the theory would suggest, be phylogenetically related.

The development of intra-epithelial clefts and spaces is not unusual. The lumina of a number of the true glands are formed within solid epithelial sprouts and buds which are the anlagen of the future ducts and tubules. Again, the vacuoles in the epithelium of the oesophagus and duodenum which were described in detail by Johnson ('10) are striking examples of this same feature of morphogenesis. In all these instances, however, the spaces ultimately form a connected system of cavities or open into a common lumen. The intra-epithelial cavities of the early thyreoid gland, on the other hand, have a very different fate. They open more or less independently of one another to the outside of the gland mass and are then invaded by the surrounding vascular mesenchyme. Such a process apparently has not been observed in the morphogenesis of any other organ and seems to be quite unique and peculiar to the thyreoid gland.

Before attempting to discuss the follicular period, the fact must be emphasized that the cavities or lumina of the thyreoid follicles are entirely independent of the earlier transient intraglandular cavities described in the present paper.

Remak's ('55) theory of the derivation of the thyreoid follicles directly from a primitive saccular thyreoid anlage has not been confirmed. In the prefollicular stages, the thyreoid is by recent investigators quite generally described as assuming the form of irregular, anastomosing 'cords' or masses of epithelium. This undoubtedly appears to be the case when sections of the gland are observed (figs. 11, 12, 13). But the reconstruction methods used in the present investigation reveal a surprisingly different condition. It is found that, as a matter of fact, in the great majority of cases the cords are illusions and in reality are merely sections of fenestrated epithelial plates longitudinally arranged.

As to the further steps in the process of morphogenesis of the follicles from these anastomosing 'cords,' widely divergent views have been held. Inasmuch as the process of follicle genesis has been found to be practically identical in Squalus acanthias with the process described by the author in the human thyreoid (Norris, '16), the reader is referred to this earlier work for the discussion of the changes noted and for a consideration of the various views presented by the literature as regards this matter.

The relative magnitude of the vascular bed which is associated with the gland, the close relationship which is established between the blood stream and the parenchymatous elements, and the comparatively small amount of arterial blood furnished to the gland are the most noteworthy conditions in connection with the blood supply of the thyreoid.

As shown in figure 14, the gland is literally suspended in a lake of blood, the blood being separated from the epithelial structure only by a single layer of epithelial cells.

7. SUMMARY

1. The thyreoid gland in Squalus acanthias makes its appearance in embryos of approximately 4 mm. in length, as a solid epithelial bud from the floor of the pharynx.

2. The bud grows caudally and becomes keel-shaped. At this stage it is joined to the pharynx by a very short and narrow stalk.

3. By the time the embryo has attained a length of 19 mm. the gland has severed its connection with the pharynx and has the form of a column with rounded ends.

4. In no case was a 'thyreoglossal duct' observed.

5. The gland soon loses its columnar form, and as it gradually flattens out dorsoventrally, becomes lozenge-shaped in outline.

6. In embryos of about 30 mm. the gland for the first time assumes in miniature the form of the adult gland. It may be divided for descriptive purposes into two portions: an anterior part (corpus), which is rhomboidal in outline and relatively thick in cross-section, and a posterior part (cauda), which is relatively thin in cross-section and has the form of a long straight bar

joined with the posterior angle of the corpus in front and terminated caudally in a pointed extremity.

7. Except for the indefinite vacuole-like spaces which are found in the thyreoid of embryos ranging from 5 to 20 mm. in length, the gland is quite solid throughout the period of its early morphogenesis.

8. At about the stage (20 mm.) when the vacuole-like spaces cease to be found, there are formed within the thicker portions of the gland a number of completely closed cavities, which in contrast to the earlier vacuole-like spaces are outlined by very definite margins. These intraglandular cavities are very tiny when they first appear, but as they increase in size they may open into one another, thus transforming the gland from a solid into a hollow organ.

9. At a later stage (28 mm.) these cavities for the first time open to the outside and are invaded by blood-vessels.

10. The process of cavity formation is accompanied or perhaps preceded by four other processes, as follows: 1) differentiation of cell boundaries; 2) changing form of the nuclei (cells); 3) changed position of the nuclei (cells); 4) cell proliferation.

11. The intraglandular cavities are regularly surrounded by epithelial plates which are two cells in thickness, so that when the cavities are opened to the outside the gland is transformed into a number of smooth epithelial plates which anastomose with each other freely and which are regularly two cells thick.

12. The primary thyreoid follicles arise directly as isolated and independent structures from the epithelial plates of the prefollicular period, by the rearrangement of cells, cell proliferation, increase in the size of the cells, and lumen formation.

13. The primary follicles appear in embryos of about 60 mm. in length and continue to be formed until the embryo attains a length of approximately 250 mm.

14. There are no secondary follicles found before 250 mm.

15. Branches of the external jugular vein invade the region of the thyreoid and begin to form a vascular plexus around the gland in embryos about 19 mm. in length.

16. The elements of this venous plexus associate themselves intimately with the parenchymatous elements. The thyreoid sinus is formed by the increase in their size and by their fusion with one another.

LITERATURE CITED

BALFOUR, F. M. 1876 The development of the elasmobranch fishes. Jour. Anat. Phys., vol. 10 (Also: A monograph on the development of elasmobranch fishes. London, 1878).
1885 The works of Francis M. Balfour. London.

BAUMGARTNER, E. A. 1915 The development of the hypophysis in Squalus acanthias. Jour. Morph., vol. 26.

BENSLEY, R. R. 1916 The normal mode of secretion in the thyroid gland. Am. Jour. Anat., vol. 19.

BORN, G. 1893 Über die Derivate der embryonalen Schlundbogen und Schlundspalten bei Säugethieren. Arch. f. mikr. Anat., Bd. 22.

CAMP, W. E. 1917 The development of the suprapericardial (postbranchial, ultimobranchial) body in Squalus acanthias. Jour. Morph., vol. 28.

FERGUSON, J. S. 1911 The anatomy of the thyroid gland of Elasmobranchs, with remarks upon the hypobranchial circulation in these fishes. Am. Jour. Anat., vol. 11.

GOODEY, T. 1910 Vestiges of the thyroid in Chlamdoselachus anguineus, Scyllium catulus, and Scyllium canicula. Anat. Anz., Bd. 36.

HIS, W. 1891 Der Tractus thyreoglossus und seine Beziehungen zum Zungenbein. Arch. f. Anat. u. Entw.

JOHNSON, F. P. 1910 The development of the mucous membrane of the oesophagus, stomach, and small intestine in the human embryo. Am. Jour. Anat., vol. 10.

MCGILL, C. 1910 The early histogenesis of striated muscle in the pig and the dogfish, Anat. Rec., vol. 4.

MINOT, C. S. 1900 On a hitherto unrecognized form of blood circulation without capillaries in the organs of vertebrata. Proc. Boston Soc. Nat. Hist., vol. 29.

MÜLLER, W. 1871 Über die Entwicklung der Schilddrüse. Jena. Zeitschr. f. Med. u. Naturw., vol. 6.

NEAL, H. V. 1914 The morphology of the eye-muscle nerves. Jour. Morph., vol. 25.

NORRIS, E. H. 1916 The morphogenesis of the follicles of the human thyroid gland. Am. Jour. Anat., vol. 20, no. 3.
1917 The early morphogenesis of the thyroid gland in Squalus acanthias. Anat. Rec., vol. 11. (Abstract of a paper presented at the meeting of the American Association of Anatomists in 1916).
1918 The early morphogenesis of the human thyroid gland. Am. Jour. Anat. (In press.)

PATTEN, W. 1917 The notochord of an East Indian scorpion. Anat. Rec., vol. 11. (Abstract of a paper presented at the meeting of the American Association of Anatomists in 1916.)

REMAK, R. 1855 Untersuchungen über die Entwickelung der Wirbelthiere. Berlin. S. 122–123.

SCAMMON, R. E. 1911 Normal plates of the development of Squalus acanthias. Hefte 12, Normentaf. d. Ent. d. Wirbeltiere. Jena.
1915 The histogenesis of the Selachian liver.
Am. Jour. Anat., vol. 17.

PLATE 1

EXPLANATION OF FIGURES

17 Left lateral view of a wax reconstruction of the pharynx of an Acanthias embryo 5 mm. long (No. 6). *E.*1 and *E.*2, ectodermal plates of the first and second gill clefts, respectively. These plates are cut away arbitrarily around their edges. *G.P.* 3, third gill pouch which has not yet fused with the ectoderm; *Nc.*, notochord; *O.Pl.*, oral plate; *T.*, thyreoid. × 75.

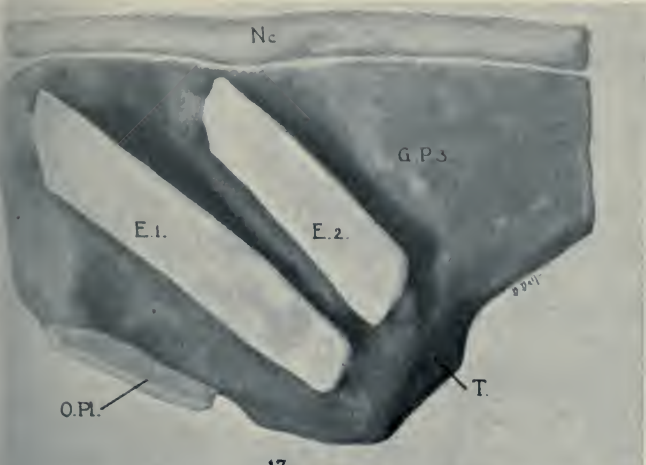
18 Right lateral view of a wax reconstruction of the pharyngeal floor of an Acanthias embryo 14 mm. long (No. 24). *G.P.*1 and *G.P*2, first and second gill pouches, respectively; *O.Pl.*, oral plate; *T.*, thyreoid. × 100.

19 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 21 mm. long (No. 34). × 100.

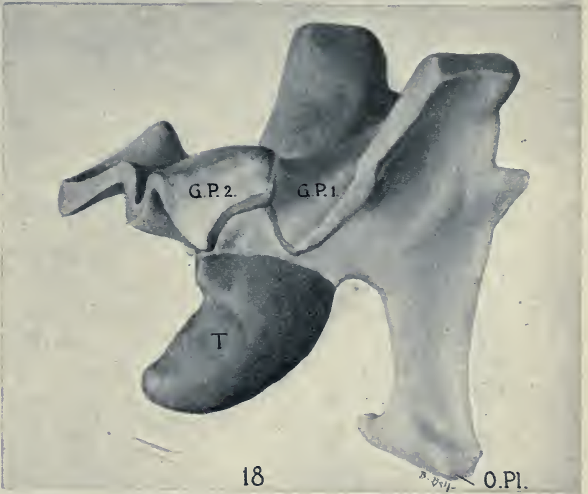
20 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 24 mm. long (No. 36). × 100.

21 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 28 mm. long (No. 38). × 100.

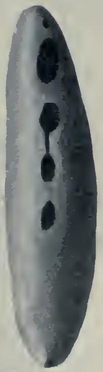
In figures 20, 21, and 22 the dark areas represent the position of intraglandular cavities whose outlines have been projected on the ventral surface of the gland.



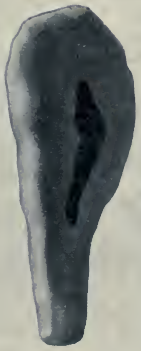
17



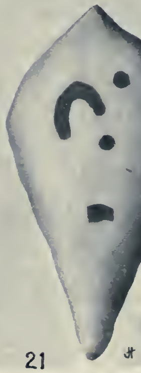
18



19



20



21

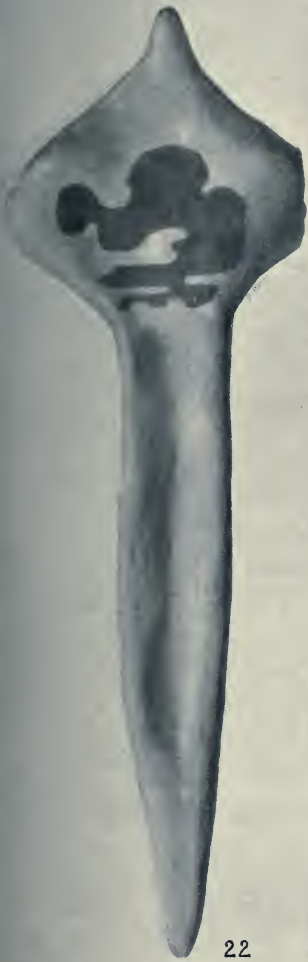
PLATE 2

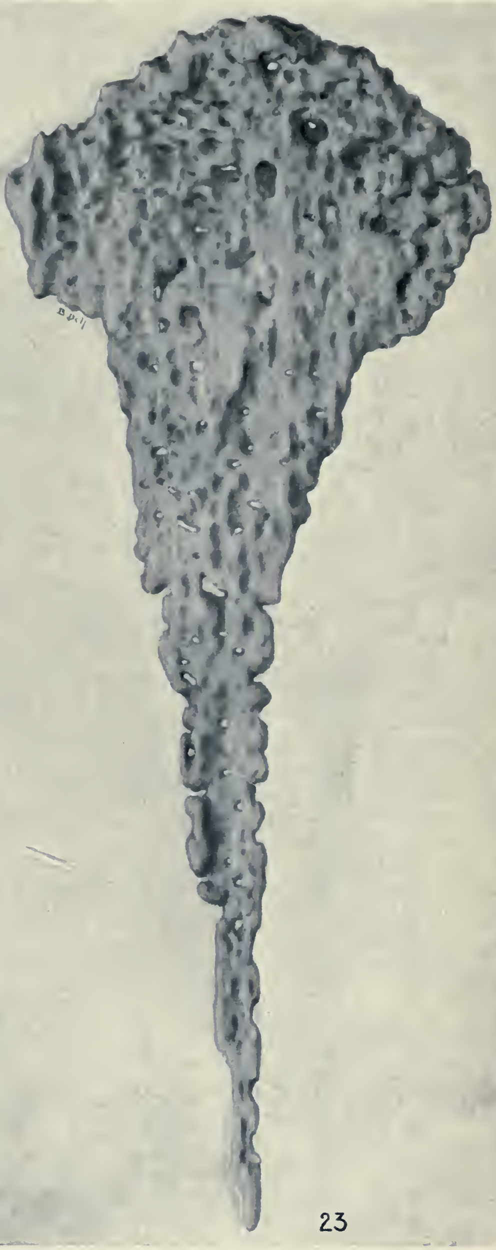
EXPLANATION OF FIGURES

22 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 36 mm. long (No. 42). The anterior rhomboidal corpus and the posterior elongated cauda are easily recognizable. The dark area on the corpus represents the position of the intraglandular cavity whose outline has been projected on the ventral surface of the gland. This cavity has opened to the outside on the dorsal aspect of the gland. Its opening is not indicated in the figure. × 100.

23 Ventral view of a wax reconstruction of the thyreoid gland of an Acanthias embryo 60 mm. long (No. 47). The roughness on the surface is due to the beginning formation of follicles in the epithelial plates. The clear areas represent perforations or fenestrae in the plates produced by the gradual separation of the forming follicles. The points at which the intraglandular cavities have opened to the outside are also seen. × 65.

E. H. NORRIS


22


23

0

9b 11

443

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAHIC SERVICE, AUGUST 15

Reprinted from THE AMERICAN JOURNAL OF
ANATOMY, Vol. 24, No. 4, November, 1918

THE EARLY MORPHOGENESIS OF THE HUMAN THYROID GLAND

EDGAR H. NORRIS

Institute of Anatomy, University of Minnesota

TWELVE FIGURES

CONTENTS

1. Introduction.. 443
2. Material and methods.. 444
3. Morphogenesis of the thyroid gland............................ 446
4. Discussion and conclusions.................................... 457
5. Summary... 463
Literature cited... 464

1. INTRODUCTION

The general facts regarding the derivation and early morphogenesis of the human thyroid gland ('median thyroid'), having been repeatedly set forth by numerous investigators, are well known. Notwithstanding the multiplicity of these observations, however, certain important features have been overlooked. Furthermore, no systematic and complete arrangement of the developmental stages has been provided and our knowledge of them is at best somewhat fragmentary and scattered. This results primarily from the lack of adequate series of successive embryonic stages hitherto available for study. In the present paper, which is based upon the study of a large and complete series of young human embryos, an attempt has been made to formulate an interpretation of the processes involved in the development of the median thyroid, as well as to present certain original observations.

This work, which suggested itself to the author during the course of an earlier investigation upon the morphogenesis of the thyroid follicles (Norris, '16), has been carried on at the Carnegie Institute of Embryology and at the University of Minnesota under the supervision of Prof. C. M. Jackson. I wish to thank Dr. Jackson for his valuable aid and criticism. I wish further to acknowledge my indebtedness to Dr. F. P. Mall for his kind assistance in providing a special grant for carrying on this study and for the many favors received while working in his laboratory.

2. MATERIALS AND METHODS

This study is based upon the collection of human embryos in the Carnegie Institute of Embryology at Baltimore and upon those in the Anatomical Laboratory of the University of Minnesota. The embryos have been variously fixed and stained, and many of the series used are in excellent condition for histological study.

The accompanying table shows the material used in this work. The seventy-two embryos used are arranged in order of their crown-rump length. An asterisk (*) following the collection number signifies that the specimen belongs in the collection of the University of Minnesota; the specimens which are not so marked are in the collection of the Carnegie Institute.

The ordinary reconstruction methods, both plastic (Born's wax-plate method) and graphic, have been utilized. The drawings from sections for reconstructions and for text-figures were made with the camera lucida or with Edinger's projection apparatus.

Table of embryos studied

SERIAL NUMBER	COLLECTION NUMBER	C. R. LENGTH	SECTION THICKNESS
		mm.	*microns*
1	1201B	2.0	10
2	391	2.0	10
3	12	2.1	10
4	1201A	2.3	5
5	779	2.75	15
6	1182B	3.0	20
7	164	3.5	20
8	186	3.5	20
9	463	3.9	10
10	136	4.0	50
11	588	4.0	15
12	786	4.0	15
13	836	4.0	15
14	963	4.0	20
15	470	4.0	10
16	148	4.3	10
17	76	4.5	20
18	1062	4.5	20
19	80	5.0	20
20	116	5.0	20
21	826	5.0	20
22	810	5.0	20
23	H6*	6.0	10
24	H61*	6.0	10
25	241	6.0	10
26	552	6.0	40
27	676	6.0	20
28	873	6.0	20
29	988	6.0	20
30	1075	6.0	20
31	800	6.5	10
32	371	6.6	10
33	2	7.0	15
34	4	7.0	10
35	18	7.0	20
36	372	7.0	10
37	383	7.0	10
38	560	7.0	40
39	617	7.0	15
40	651	7.0	40
41	1091	7.2	20
42	H13*	7.5	15

Table of embryos studied—Continued

SERIAL NUMBER	COLLECTION NUMBER	C. R. LENGTH	SECTION THICKNESS
		mm.	*microns*
43	221	7.5	20
44	1354	7.85	20
45	113	8.0	10
46	1006	8.0	20
47	389	8.0	20
48	792	8.0	20
49	163	9.0	20
50	388	9.0	25
51	721	9.0	15
52	887	9.0	40
53	422	9.0	40
54	452	9.0	40
55	1197	10.0	20
56	114	10.0	10
57	397	10.0	10
58	623	10.1	20
59	109	10.5	20
60	H60*	11.0	20
61	H68*	11.0	15
62	353	11.0	10
63	916	11.0	40
64	544	11.5	40
65	1121	11.8	40
66	H134*	12.0	20
67	175	13.0	20
68	695	13.5	10
69	144	14.0	40
70	H1*	15.0	12
71	H23*	15.0	10
72	H18*	15.5	10

3. MORPHOGENESIS OF THE THYROID GLAND

For descriptive purposes the morphogenesis of the thyroid gland is readily divisible into eight stages, as follows:

1. Pre-anlage stage.
2. Early anlage stage.
3. Early growth stage.
4. Beginning separation stage.
5. Complete separation stage.
6. Cavity formation stage.
7. Plate stage.
8. Follicular stage.

This more or less arbitrary division of the developmental process is based entirely upon the changing form and structure of the gland, irrespective of the age and size of the embryo. The relations of these various stages are best set forth in the accompanying diagram (fig. 1).

1. Pre-anlage stage. This stage may be defined as that period in embryonal development between the time of the formation of the entodermal pharynx and the appearance of the thyroid anlage (fig. 1, *a*). This stage precedes those represented in the present series.

2. Early anlage stage. The anlage of the thyroid gland is well formed even in the earliest members of the present series. When first recognizable, the anlage has the form of a fairly definite and rather extensive though shallow evagination from that portion of the mesobranchial region of the pharyngeal floor which lies between the ventral extremities of the first two pairs of gill pouches (figs. 1*b*, 2, 3). The cavity of the thyroid pouch or diverticulum thus formed is encroached upon and partially obliterated from below by a localized thickening of the epithelium in its floor. Such a condition occurs in nine of the embryos ranging from 2 to 4 mm. in length (Nos. 1, 2, 3, 4, 5, 6, 7, 8, and 12 of the present series).

3. Early growth stage. Whereas the gland in the preceding stage is represented by a single typical form, the early growth stage presents three distinct types (figs. 1,*c*; 1,*d*; 1,*e*). Although these three types are quite different in form, they all result from one process, that of growth. Being derived from a common parent and produced by a like process, the three types apparently owe their differences in form to various distributions of the growth activity in the anlage.

The first type presents the form of a solid pyriform globose bud suspended from the floor of the pharynx by a short, solid neck (fig. 1, *c*). There is only a slight depression remaining to indicate the previous existence of a thyroid pouch. This type occurs in Nos. 11, 15, 18, and 26 of the present series.

The second type differs from the first in that the suspending stalk is hollow and tube-like rather than solid (figs. 1,*d*; 8).

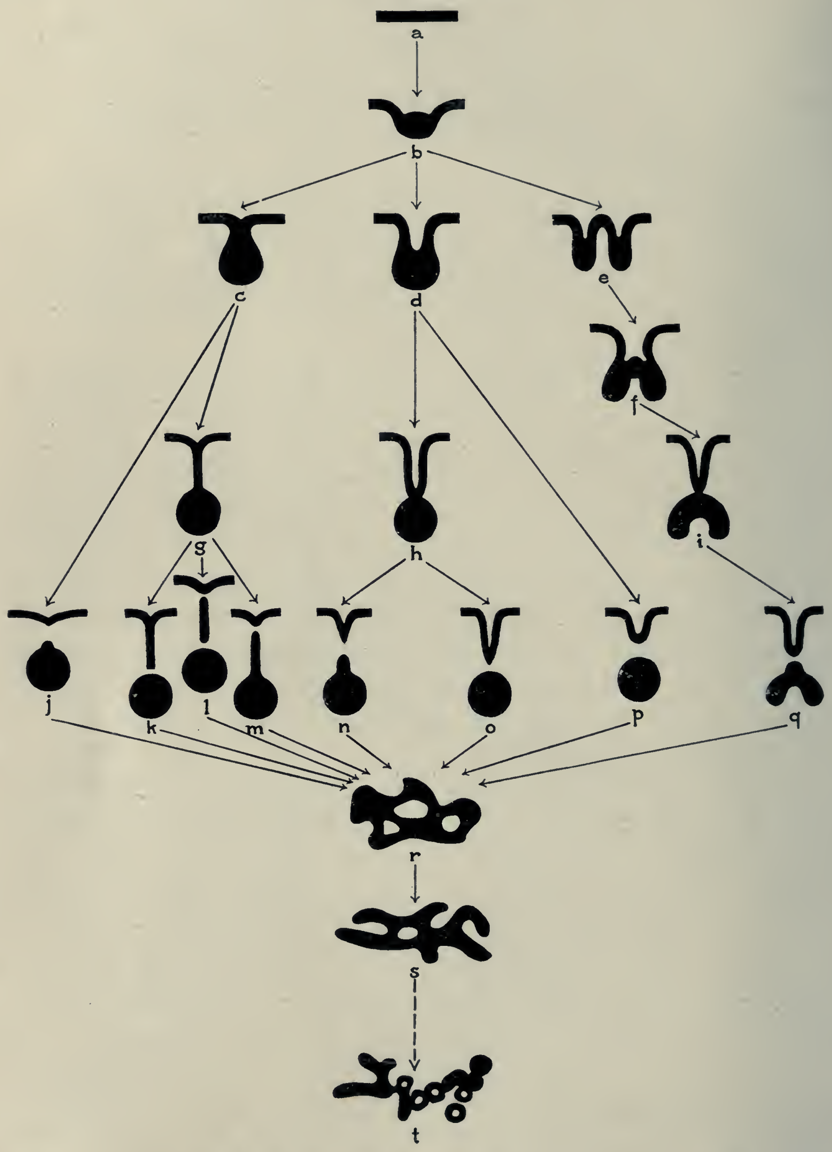


Fig. 1 A schematic interpretation of the process of morphogenesis of the human thyroid gland. With but two exceptions (*d* and *g*), each form included in this arrangement is a semidiagrammatic drawing of the condition found in sections of embryos. The parts in solid black represent the epithelium of the pharyngeal floor and the thyroid gland in the various phases of its derivation. For a description of the individual units see text. *a*, pre-anlage stage; *b*, early

This condition was found in Nos. 13, 14, and 29 of the present series.

The third type is widely different from either of the preceding in that in place of a single bud the gland is already clearly bilobed. Two lateral masses, each suspended from the pharyngeal floor by a short, hollow stalk, joined one with the other by a mound-like fold of epithelium and separated by intervening mesenchyme, represent the thyroid gland (figs. 1,*e*; 4). This type of gland was found in but one specimen (No. 17) of the present series.

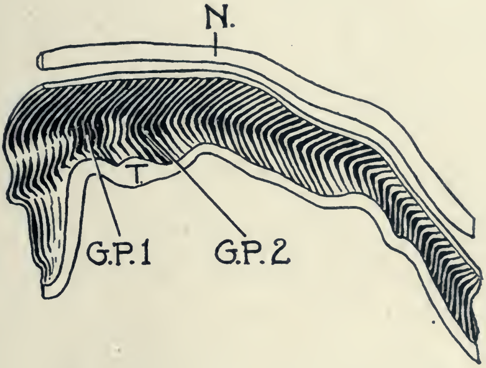
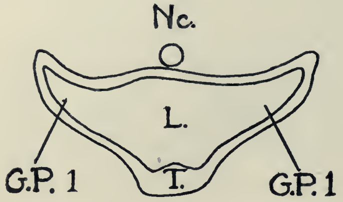


Fig. 2 Graphic reconstruction of a portion of the foregut of a human embryo 2 mm. long (No. 1) to demonstrate the form and position of the thyroid anlage (*T.*). *N.*, notochord; *G.P.1*, first gill pouch; *G.P.2*, second gill pouch. × 90.

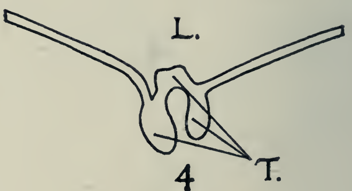
4. Beginning separation stage. This stage, like the last preceding, is represented by three types as regards form. These are apparently later developmental derivatives, one from each of the three preceding types. Each is probably produced by a

anlage stage, *c, d, e,* early growth stage, *f,* a form included to show the probable relationship of *e* and *i*; *g, h, i,* beginning separation stage; *j, k, l, m, n, o, p, q,* complete separation stage; *r,* cavity formation stage. The figure shows a cross-section of a gland having an irregular outline and three closed cavities within. (These cavities are entirely different from the follicle cavities, which appear later); *s,* plate stage. The figure shows the cross-sectional appearance of the gland after the primary cavities have begun to open to the outside; *t,* follicular stage. The figure shows a cross-section of an irregular two-celled epithelial plate in which follicles are being formed. One follicle has been completely separated off from the plate.

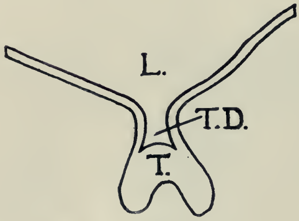
similar process—the lengthening of the suspending neck or stalk. The first type (fig. 1, *g*), in which the stalk is represented by a solid cord, is hypothetical (not having been found in the series studied), but is included to set forth more clearly the probable relations of the earlier and later stages. The second type (fig. 1, *h*) possesses a long hollow stalk, and is found in embryo No. 35. It differs from the third type (figs. 1,*i*; 6) only in the fact that the body of the latter is bilobed. The third type occurs in



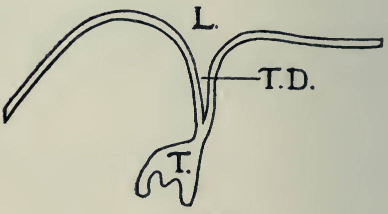
3



4



5



6

Fig. 3 Outline drawing of the epithelial lining of the pharynx of a human embryo 2 mm. long (No. 1) as seen in cross-section. *L.*, lumen of pharynx; *Nc.*, notochord; *T.*, thyroid anlage; *G.P.1*, first gill pouch. × 100.

Fig. 4 Outline drawing of the epithelial lining of the pharyngeal floor of a human embryo 4.5 mm. long (No. 17) as seen in cross-section. The section is taken through the thyroid gland which appears as a bilobed structure in this specimen. × 100.

Fig. 5 Outline drawing of the epithelial lining of the pharyngeal floor of a human embryo 6 mm. long (No. 28) as seen in cross-section. The section is taken through the thyroid gland which appears as a biloed b structure suspended from the pharyngeal floor by a short hollow stalk. (*T. D.*, thyreoglossal duct) × 100.

Fig. 6 Outline drawing of the epithelial lining of the pharyngeal floor of a human embryo 6.5 mm. long (No. 31) as seen in sagittal section. This section is taken through the thyroid gland which appears as an irregularly bilobed structure suspended from the pharyngeal floor by a long hollow stalk. × 100.

embryo No. 28. Between this third type and its predecessor, an extra stage, representing the condition found in No. 31, has been included in the diagram to indicate the way in which the previously bilobed gland probably comes to be suspended by a single hollow stalk (figs. 1,*f*; 5).

5. Complete separation stage. Apparently the thyroid may sever its connection with the pharynx in embryos of various lengths. The earliest instance in which the gland was found free is in an embryo of 3.9 mm. (No. 9). While the oldest specimen which shows the gland still attached to the pharyngeal floor in this series was found in the case of an embryo measuring 7 mm. in length (No. 35). There also appears to be great variability in the form of the gland at the time at which its independence is established. This is well brought out in figure 1. It will be noted that certain of the glands have been cut off close up to the pharynx, while others have been set free at a much greater distance from the floor of the pharynx by the division of the long suspending stalk. There are four ways in which the stalk may be divided. It may break at the proximal end (pharyngeal) (figs. 1,*j*; 1,*m*; 1,*p*), or at the distal end (glandular) (figs. 1,*k*; 1,*o*), or at both (fig. 1, *l*); or it may divide midway between the pharynx and the gland-mass so that a portion is left in connection with the pharyngeal floor and the other part attached to the gland (figs. 1,*n*; 1,*q*).

Before going further with a description of the form of the gland it is desirable to consider in detail the histologic structure of the organ during these earlier stages. As shown in figure 7, no cell boundaries are found in the earliest members of the series. The cytoplasm has a clear, almost hyaline appearance and only infrequently shows a very few fine scattered granules. The nuclei are fairly typical. They are large ovoidal bodies having very distinct nuclear membranes. In general, the nuclei are so arranged that their long axes are nearly perpendicular to the surfaces of the anlage. Each nucleus contains three or four large smooth chromatin blocks (karyosomes) which are placed in an almost structureless karyoplasm and lie either in apposition with the nuclear membrane or apparently free in the nuclear sap. A few fine linin fibrils may be made out passing through the karyoplasm and joining two or more of the chromatin masses (fig. 7).

In embryos of 4 mm. (fig. 8) certain other structural differences may be observed. Cell boundaries are beginning to be differentiated and are easily distinguishable in some parts of the anlage. The nuclei instead of being ovoidal or ellipsoidal are much more nearly spherical in outline. The nuclei are also more chromatic, staining more deeply than in the earlier stages.

6. Cavity formation stage. In figure 1 the early thyroid gland has been somewhat diagrammatically represented as having a globular or simple bilobed form and a nearly uniform size. In general this is a correct representation, although there is considerable individual variation in both respects. This is true not only in the beginning separation stage, but especially after the thyroid has become cut off from the pharynx. Through rapid growth the gland then increases in size and assumes the form of a thick, irregular plate-like mass which is more or less definitely bilobed (fig. 9). This gland-mass or plate, which averages five or six cells thick in cross-section, is placed ventral to the trachea in a plane parallel with the long axis of the body.

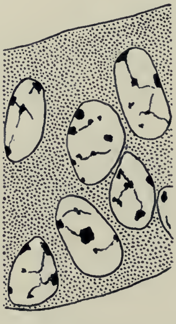


Fig. 7 Small portion of a cross-section of the thyroid gland in a human embryo 2 mm. long (No. 1) highly magnified to show structure. The upper side of the figure is the side next to the lumen of the pharynx. × 1350.

It should be carefully noted that throughout all the preceding stages (exclusive of the pre-anlage stage and the plate stage just mentioned) the gland has existed as a solid, bud-like diverticulum, at first suspended from the floor of the pharynx by a solid or hollow stalk, but later becoming detached and free. In embryos of about 7 mm. there appear within the gland mass a number of completely closed cavities. These intraglandular cavities are at first only tiny clefts, appearing very much as though the cells around them had but pulled a little apart. At first the cavities are very small, but they soon increase in size and are thus brought more closely into relation one with another. Nothing more can be said by way of generalization regarding their size, shape, or arrangement. Great differences are found in these respects in cavities of the same gland as well as in glands at different stages of development. In every case the cavity is outlined by a very distinct and sharp margin, and in no case is there any visible content within.

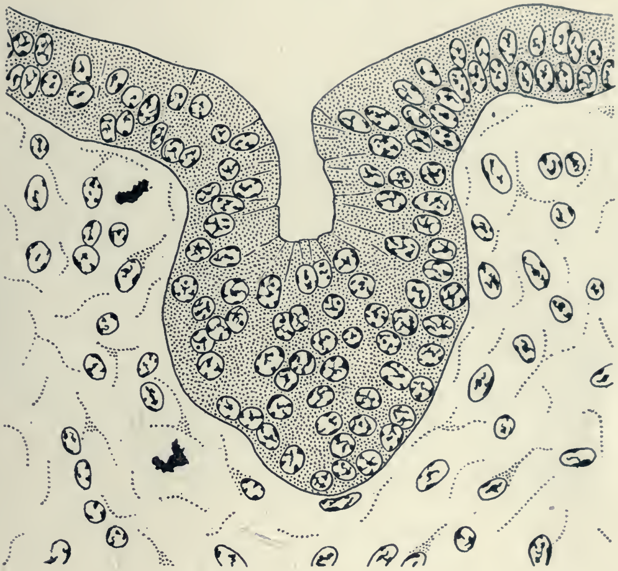


Fig. 8 A cross-section of the pharyngeal floor and the attached thyroid gland in a human embryo 4 mm. long (No. 13), magnified to show structure. × 500.

It is important to note that these spaces are apparently quite independent of any external conditions, and in these early stages in no case open to the outside. Furthermore, as shown in figures 1, *r*, 10, and 11, they develop independently of one another as isolated spaces, and only secondarily may become confluent. At a later stage these cavities, for the first time, open to the outside and are then invaded by the adjacent vascular mesenchyme (fig. 1, *s*). They develop only in those parts of the gland-mass which have attained considerable thickness, and have never been observed in those parts which do not exceed two cells in thickness. Cavities are found in thirty of the specimens studied (Nos. 22, 25, 27, 32, 36, 37, 38, 40, 41, 42, 44, 46, 48, 49, 50, 51, 55, 56, 57, 58, 59, 60, 61, 62, 65, 67, 68, 70, 71, 72).

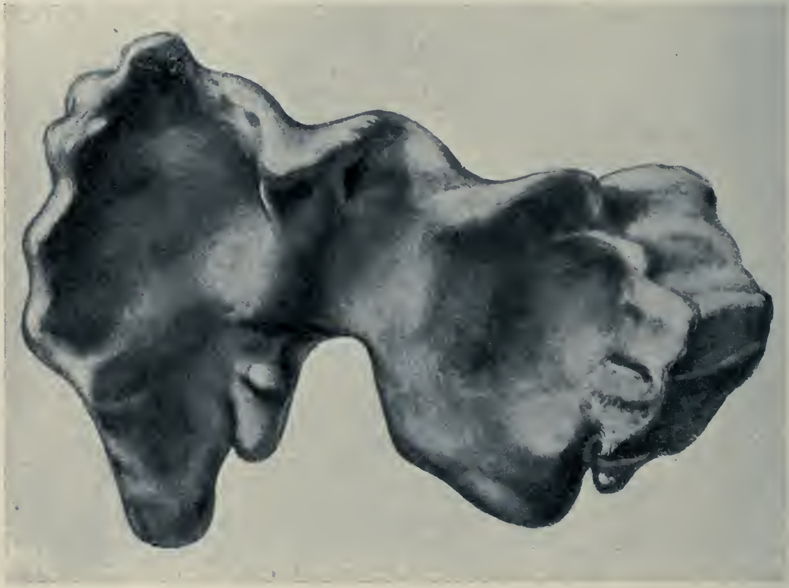


Fig. 9 Wax reconstruction of the thyroid gland in a human embryo 7.5 mm. long (No. 42). The bilobed condition of the gland is clearly shown. The gland, whose general surface is smooth, presents thick and thin parts. In the thick portion near the center of the upper border a cavity which has opened to the outside is seen. × 180.

7. *Plate stage.* As a result of the development of these intraglandular cavities, the gland-mass is transformed from a solid

body into one which is hollow and whose cavities are surrounded by epithelial plates two cells in thickness. These cavities do not long persist, but, as above mentioned, very soon open to the outside and are invaded by the surrounding vascular mesenchyme. As a result of this process, the gland comes to be made up of a progressively larger number of smooth, two-celled, epithelial plates which anastomose freely with each other and ultimately come to form an extremely complex structure. The gland in this condition, as it is found at the end of the plate stage, is not figured in the present paper since it has been fully described and figured in an earlier contribution (Norris, '16).

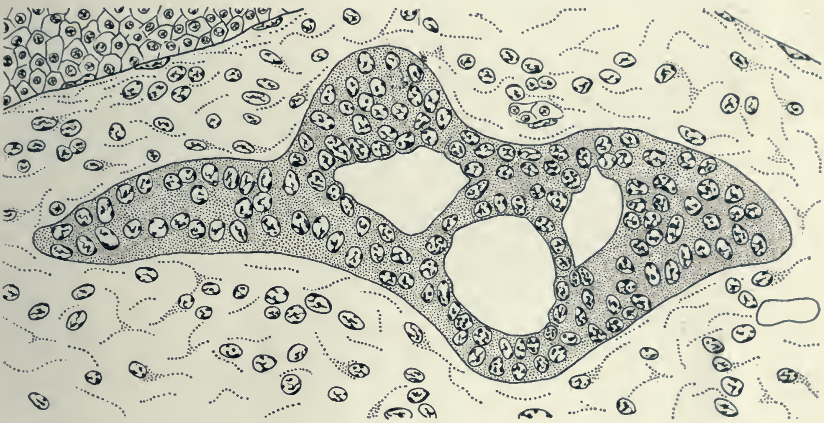


Fig. 10 Cross-section of the thyroid gland in a human embryo 7 mm. long (No. 37), magnified to show structure and the presence of intraglandular cavities. × 400.

This process of cavity formation is not the only method by which the epithelial plates are formed. It is, however, the process apparently involved in the initial breaking up of the gland-mass, and continues for only a short time. It is superseded by the derivation of plates from those already formed, by processes of budding and growth. Apparently the upper poles of the lateral lobes of the adult gland are formed from those secondary plates which grow cephalad. This is believed to be so because, notwithstanding the fact that cavities have never been observed in this region, there is a progressive increase in the length of the lateral lobes from stage to stage. These plates are regularly two cells thick and are formed independent of any intraglandular cavities. The writer has observed the development of cavities only in those thickened portions of the early gland-mass which in general apparently correspond to the region of the isthmus and lower one-third of the lateral lobes of the adult gland. Many of these points are well illustrated in figure 12, which is a model of the thyroid gland and associated structures of an embryo 15.5 mm. long (No. 72). This model not only serves to set forth the structure of the gland while the plates are being formed, but also represents the important stage in which the lateral anlage has just fused with the median.

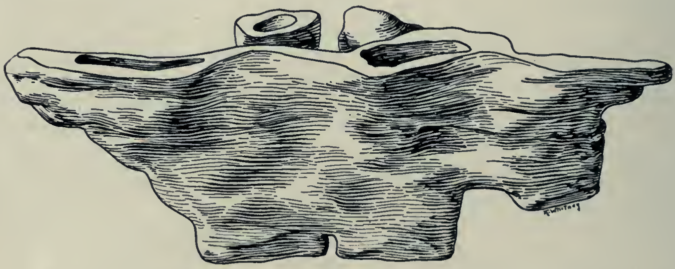


Fig. 11 Wax reconstruction of the thyroid gland in a human embryo 11 mm. long (No. 60). The upper portion of the model has been removed in order to demonstrate the relations to the gland mass to the appearances found in cross-section. Three cavities are shown in the thicker portions of the gland. × 150.

8. Follicular stage. The developing thyroid follicles gradually replace the epithelial plates in which they are formed. Figures 1, *s*, and 1, *t*, indicate the relation of these two stages. It is unnecessary to describe the process of follicle genesis further than to emphasize the fact that the cavities or lumina of the thyroid follicles are entirely independent of the earlier transient intraglandular cavities described in the present paper, which in turn are independent of the primitive lumen of the thyreoglossal duct. For a description of the gland structure from the end of the prefollicular period through the remaining period of fetal development the reader is referred to an earlier publication, (Norris, '16).

4. DISCUSSION AND CONCLUSIONS

The remarkably early appearance of the thyroid in the human embryo, as has been pointed out by various observers, is a point of unusual interest. Thus the anlage of the median thyroid is well formed and easily recognizable in the Kroemer-Pfannenstiel (1.38 mm.) and in the Rob. Meyer embryo No. 335 (1.70 mm.) (Grosser, '12). Each of these is somewhat younger than the earliest members of the present series and indicate that the thyroid appears much earlier than is shown in the Normentafel (Keibel and Elze, '08) (2.5 mm.). What the meaning of this early development of the gland is cannot as yet be said. It may

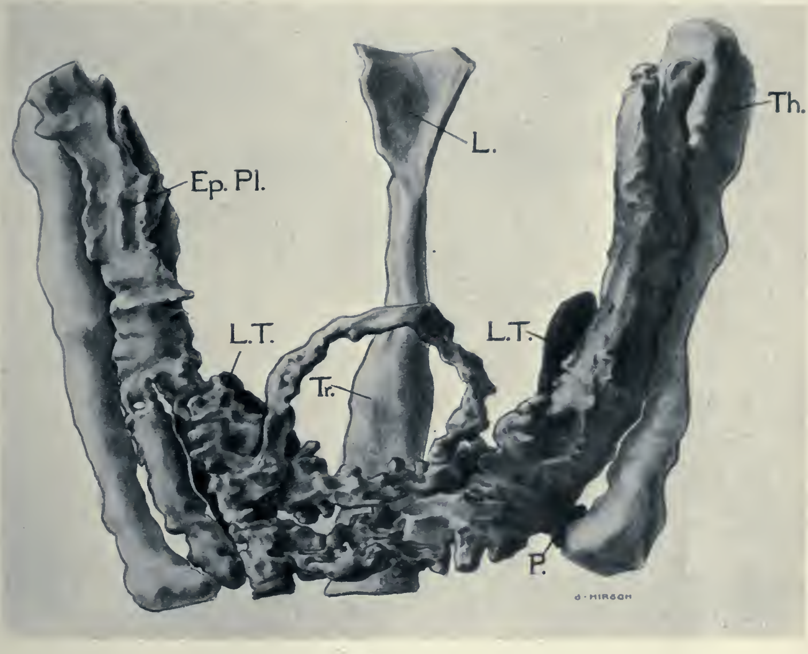


Fig. 12 Wax reconstruction of the thyroid gland and associated structures in a human embryo 15.5 mm. long (No. 72). The thyroid gland, which is in the plate stage, simulates rather closely the gross form of the adult organ—there being two lateral lobes joined in front of the trachea by an isthmus, which in this specimen is divided into three parts. *Ep.Pl.*, epithelial plate of the thyroid gland; *L.*, larynx; *L.T.*, lateral thyroid; P., parathyroid; *Th.*, thymus; *Tr.*, trachea. × 80.

be significant of the fundamental phylogenetic rôle of the structure, or of its great physiologic import, or possibly, on the other hand, may only be an expression of the general vertebrate tendency of craniocaudal development. In the latter case, just as the primitive body segments develop in regular succession cephalocaudally, and just as the lateral pharyngeal pouches appear successively in the same direction, so it might be supposed that the thyroid anlage, which lies cephalad to the region from which the lungs, liver, and pancreas arise, would appear somewhat earlier than these.

Embryo No. 2 of this series is the specimen described by Dandy ('10). Although Dandy states specifically that no thyroid anlage has made its appearance in this embryo, in describing the foregut he says: "These pouches (referring to the first pair of pharyngeal pouches) are continuous ventrally and unite to form a ventral pouch." This 'ventral pouch' apparently represents the anlage of the median thyroid, as has been noted by Grosser ('12).

The relatively large area of the pharyngeal floor which is involved in the primitive thyroid anlage is a point worthy of notice. This ventral thyroid pouch is very like the first lateral pharyngeal pouch of this same stage, both as regards size and form. As pointed out in the preceding section, this pouch has the form of a fairly definite and rather extensive, though at first shallow evagination. The floor of this outpouching becomes thickened at an early stage. Since this condition has been so regularly observed in the early specimens studied in this series (Nos. 1, 2, 3, 4, 5, 6, 7, 8, and 12), and since those isolated cases found in the literature (His, '85, Broman, '96, Sudler, '02, and the Rob. Meyer and Kroemer-Pfannenstiel embryos—Grosser, '12) likewise accord with this description, one is probably justified in concluding that this structure is the typical thyroid anlage for the human species.

The apparently discordant descriptions of the early thyroid gland found in the literature are probably not due to inaccurate observations. According to the scheme shown in figure 1, they are explainable rather as normal variations, the successive stages being represented by a progressively larger number of forms. The apparent discord has arisen because only more or less isolated cases have been studied by a single observer. From the investigation of the present large series it has been possible to arrange the various forms found into a system on the basis of which the descriptions afforded by the literature may be harmoniously interpreted. Thus, with but few exceptions, forms similar to all those included in figure 1 have been described, more or less adequately, by earlier observers. But no one has undertaken heretofore to correlate these observations, and consequently an appreciation of their relative significance has been impossible.

According to the present interpretation, the thyroid gland may take one of three courses of development following the anlage stage. In the first type it assumes the form of a solid globose bud connected to the pharynx by a short, solid stalk (fig. 1, *c*). Apparently such a condition was found in embryos No. 13 and No. 14 of the Normentafel series (Keibel and Elze, '08), and in the 3-mm. embryo described by Hammar ('02). In the second type the gland presents an appearance similar to that just described, differing only in that it is suspended by a hollow stalk (fig. 1, *d*). This is apparently the form of the gland found by Ingalls ('07), Thompson ('07), Kingsbury ('15), and Johnson ('17). The third type, which is clearly bilobed and gives the appearance of a double gland (figs. 1,*c*; 1,*f*), is of special interest. Apparently such a structure was also found in embryo No. 16 of the Normentafel series and in the 5-mm. embryo described by Hammar ('02). A peculiar interest attaches to this form from the phylogenetic point of view. The finding would seem to support the suggestion by Patten ('17) that the median thyroid may be the combined representative of the two glands found connected with the pharynx in certain of the lower forms.

In the beginning separation stage, which is also represented by three types corresponding to those of the preceding stage, the principal changes are found in the stalk by which the gland is suspended. The stalk becomes elongated or drawn out, so that the gland is removed from the pharyngeal floor for a greater distance than in the earlier stages. The first type of this stage (fig. 1, *g*), which is hypothetical so far as the present series is concerned, apparently corresponds to the condition found by Keibel and Elze ('08) in embryo No. 21 of the Normentafel series. No previously described cases have been found which correspond to the second type represented by figure 1, *h*. There are several cases in the literature in which the thyroid is described as having a form similar in part to both figures 1, *g*, and 1, *i*. That is, the gland which is definitely bilobed is suspended from the pharyngeal floor by a solid stalk rather than by a tubular connection. Such a condition was apparently found in embryos Nos. 20, 22, 23, and 25 of the Normentafel series, and by His ('85) in embryo Sl (the thyroid of which, however, he described as having the form of a double epithelial vesicle rather than that of a solid bilobed gland), and by His ('91) in embryo Rn.

As indicated in figure 1 (*j, k, l, m, n, o, p, q*) the complete separation stage is represented by a variety of forms which differ from one another, as has been already pointed out, as to the form of the gland at the time of its separation from the pharyngeal floor and upon the ways in which the thyreoglossal duct divides. The variations in this stage serve to explain the developmental processes involved in those cases in which a pyramidal lobe is found (described by His ('91) as developing from the 'thyreoglossal duct'), as well as those cases in which no such lobe is present (fig. 1, *j, k, l, m, o, p*). The development of the so-called 'lingual rests' and 'suprahyoid bodies' of Streckeisen ('86) are also explainable as arising from conditions similar to those represented by figure 1, (*k, l, n, o, p, q*). The variety of forms which the foramen caecum may assume may likewise be accounted for (figs. 1, *j*–1, *q*). Kanthack ('91), "in an article of slight value" (Minot, '92), has denied that the foramen caecum, the pyramidal lobe of the thyroid, and the thyroid 'rests' which have been found in postnatal life, are related to the morphogenesis of the thyroid gland as was first pointed out by His ('91).

After the gland has established its independence from the pharynx its structure is again altered, as described above, by the development of completely closed cavities within the thicker parts of the gland mass. These cavities, although not previously described in the human thyroid, apparently correspond to the 'lumen' which Keibel and Elze ('08) observed in the thyroid of four specimens in the Normentafel series (Nos. 26, 28, 41, 45). They merely mention the presence of these spaces.

Grosser ('12) states that "the thyroid anlage, before its separation from the pharynx, becomes bilobed with a divided lumen;" but he cites no specific cases in which this condition was found. Moreover, this generalization receives no support from my studies nor from the researches of other investigators. Since the gland has been regularly found to be solid up to the time of its separation from the pharynx and since the thyreoglossal duct (that part of it which remains attached to the gland) at this stage of complete separation has in no case in my series presented a lumen, it is evident that these cavities develop quite independently of the original thyroid pouch. Further evidence in favor of this conclusion may be drawn from the fact that a number of these closed cavities or spaces of various sizes have been found in different stages of development not only in many of the glands (Nos. 22, 25, 27, 32, 33, 36, 37, 38, 40, 41, 42, 44, 46, 48, 50, 51, 55, 56, 57, 58, 59, 60, 61, 62, 65, 67, 68, 70, 71, 72), but also in different parts of the gland in the same specimen.

The cavities which I first observed in the human thyroid have been subsequently found in the thyroid of Squalus acanthias embryos (Norris, '17, '18). In these fish embryos the cavities appear at a stage in the glandular development which exactly corresponds to the stage of their genesis in the human thyroid. Moreover, the rôle they play in the two forms is apparently identical. Born ('83), in describing the thyroid glands of pig embryos 7 mm. long, mentions the presence of lumina in the lateral end of the gland mass. He does not include any description of these lumina, but they probably correspond to the cavities described in this paper. Since finding these intraglandular spaces in the thyroids of human embryos and in those of Squalus acanthias embryos, the author has made a casual study of a number of intermediate forms (pig, sheep, dog, rabbit, pigeon) and has found similar cavities present in the thyroid of each form investigated. It is hoped that these observations may be extended and presented in a subsequent publication. The existence of this same feature of morphogenesis in the thyroid glands of two animal groups so far removed from one another as fish and man, as well as in the glands of a number of intermediate forms, would probably justify the conclusion that this is a fundamental feature of thyroid development.

Any attempt to interpret these early intraglandular spaces either on the basis of their immediate or general biologic significance, although interesting, can be at best only speculative. It might be thought that the immediate purpose and significance of these cavities is to be found in the formation of the two-celled plates from which the follicles are later to be derived. On the other hand, if the vertebrate thyroid is the phylogenetic representative of a true externally secreting gland, it might be suggested that these cavities appear in response to a tendency to reproduce the ancient lumen or duct of the ancestral gland. This theory is attractive, inasmuch as it permits of harmonizing the known facts, both anatomic and physiologic, regarding the endocrine function of the thyroid. From this point of view the hypothesis and conclusion of Bensley ('16), "that the thyroid cell represents a true reversal of polarity," would be quite unnecessary. On the contrary, this phylogenetic theory would offer a plausible explanation of the present structure of the gland and its endocrine function without making it essential to hypothesize a reversal of cellular polarity.

It might be objected that the fact that these intraglandular spaces have no relation to the lumen of the thyreoglossal duct would appear to be a serious obstacle to the theory just advanced. But such a contention is not as formidable as it might seem at first sight, when it is considered that there are no specific cases described in which the lumen of the thyreoglossal duct is continuous with a cavity in the gland-mass proper. In other words, since the lumen of the duct does not extend into the body of the gland, any cavities found there must be morphologically independent of it, although they may, as the theory would suggest, be phylogenetically related.

The development of intra-epithelial clefts and spaces is not an unusual occurrence. The lumina of a number of the true glands are formed within solid epithelial sprouts and buds which are the anlages of the future ducts and tubules. The vacuoles in the epithelium of the oesophagus and duodenum which were described by Johnson ('10) and earlier by Keibel and Elze ('08), are also striking examples of this same feature of morphogenesis. In all these instances, however, the spaces ultimately form a connected system of cavities or open into a common lumen. The intra-epithelial cavities of the early thyroid gland, on the other hand, have a very different fate. They open more or less independently of one another to the outside of the gland-mass and are then invaded by the surrounding vascular mesenchyme. Such a process apparently has not been observed in the morphogenesis of any other organ and seems to be quite unique and peculiar to the thyroid gland.

5. SUMMARY

1. The morphogenesis of the human thyroid gland is divisible into the following stages: 1) pre-anlage; 2) early anlage; 3) early growth; 4) beginning separation; 5) complete separation; 6) cavity formation; 7) plate stage; 8) follicular stage.

2. The typical anlage of the human thyroid gland apparently has the form of a fairly definite and rather extensive, though shallow evagination from the pharyngeal floor between the ventral extremities of the first two pairs of gill pouches. The floor of this thyroid pouch becomes thickened at an early stage.

3. Following the anlage stage, the successive stages are represented by a progressively larger number of forms. These types differ from one another both as regards the form of the body of the gland and as regards the condition of the stalk (thyreoglossal duct) by which it is suspended from the pharyngeal floor.

4. A definite thyreoglossal duct may or may not be present during the development of a gland. The duct may be hollow or solid and of variable length. In those cases where no thyreoglossal duct is formed, the gland is constricted off close up to the pharyngeal floor.

5. The time at which the thyroid severs its connection with the pharynx is variable—the period ranging, as regards length of embryo, from 3.9 mm. to 7 mm.

6. In embryos of approximately 7 mm. there appear within the previously solid body of the thyroid a number of completely closed cavities. These intraglandular cavities, which at first are small, may increase in size, and sometimes open one into another. Finally they open to the outside, and are then invaded by vascular mesenchyme. A similar process has been observed in the early embryonic thyroid of other forms, including the pig, sheep, dog, pigeon, and dogfish.

7. As a result of the development of these intraglandular cavities, the gland-mass is transformed from a solid body into one which is hollow and whose cavities are surrounded by epithelial plates two cells in thickness. So that when the cavities open to the outside the gland undergoes a corresponding structural modification, being changed from a hollow organ to one made up of a complex network of epithelial plates (plate stage).

8. The transient intraglandular cavities are entirely independent of the follicle lumina, which appear later as described in an earlier paper (Norris, '16).

LITERATURE CITED

Bensley, R. R. 1916 The normal mode of secretion in the thyroid gland. Am. Jour. Anat., vol. 19.

Born, G. 1893 Über die Derivate der embryonalen Schlundbogen und Schlundspaten bei Säugetieren. Arch. f. mikr. Anat., Bd. 22.

Broman, I. 1896 Beschreibung eines menschlichen Embryos von 3 mm. Länge mit spezieller Bemerkung über die bei demselben befindlichen Hirnfalten. Morph. Arb., Jena, Bd. 5.

Dandy, W. E. 1910 A human embryo with seven pairs of somites. Am. Jour. Anat., vol. 10.

Grosser, O. 1912 The development of the pharynx and of the organs of respiration. Keibel and Mall's Human Embryology, vol. 2, pp. 446–497.

Hammar, J. A. 1902 Studien über die Entwicklung des Vorderdarms und einiger angrenzenden Organe. Arch. f. mikr. Anat., Bd. 59.

His, W. 1885 Bildung der Schilddrüsenanlage. In Anatomie menschlicher Embryonen.

1891 Der Tractus thyreoglossus und seine Beziehungen zum Zungenbein. Arch. f. Anat. u. Entw.

INGALLS, N. W. 1907 Beschreibung eines menschlichen Embryos von 4.9 mm. Arch. f. mikr. Anat., Bd. 70.

JOHNSON, F. P. 1910 The development of the mucous membrane of the oesophagus, stomach, and small intestine in the human embryo. Am. Jour. Anat., vol. 10.

1916 A human embryo of twenty-four pairs of somites. Publication 226 of the Carnegie Institute of Washington.

KANTHACK, A. A. 1891 The thyreo-glossal duct. Jour. Anat. and Physiol., vol. 25.

KEIBEL, F., AND ELZE, C. 1908 Normentafeln zur Entwicklungsgeschichte der Wirbeltiere. S. 91–151. Jena.

KINGSBURY, B. F. 1915 The development of the human pharynx. Am. Jour. Anat., vol. 18.

KROEMER-PFANNENSTIEL According to Grosser ('12) in Keibel and Mall's Human Embryology, vol. 2, p. 447.

MALL, F. P. 1891 A human embryo twenty-six days old. Jour. Morph., vol. 5.

MEYER, R., according to Grosser ('12) in Keibel and Mall's Human Embryology, vol. 2, pp. 447 and 449.

MINOT, C. S. 1892 Human embryology.

NORRIS, E. H. 1916 The morphogenesis of the follicles in the human thyroid gland. Am. Jour. Anat., vol. 20.

1917 The early morphogenesis of the thyroid gland in Squalus acanthias. Anat. Rec., vol. 11. (Abstract of a paper presented at the meeting of the American Association of Anatomists in December, 1916.)

1918 The morphogenesis of the thyroid gland in Squalus acanthias. Jour. Morph., vol. 31.

PATTEN, W. 1917 The notochord of an East Indian scorpion. Anat. Rec., vol. 11. (Abstract of a paper presented at the meeting of the American Association of Anatomists in December, 1916.)

STRECKEISEN, A. 1886 Beiträge zur Morphologie der Schilddrüse. Virchow's Archiv, Bd. 103.

SUDLER, M. T. 1902 The development of the nose, and of the pharynx and its derivatives in man. Am. Jour. Anat., vol. 1.

THOMPSON, P. 1907 Description of a human embryo of twenty-three paired somites. Jour. of Anat. and Physiol., vol. 41.

No. 12.

CYCLIC CHANGES IN THE INTERSTITIAL CELLS OF THE OVARY AND TESTIS IN THE WOODCHUCK (MARMOTA MONAX)

Andrew T. Rasmussen

(From the Departments of Histology and Embryology, and of Physiology,
Cornell University, Ithaca, New York; and The Institute of
Anatomy, University of Minnesota, Minneapolis, Minnesota)

Four plates (36 figures)

CONTENTS

Introduction 354
Cyclic changes in the interstitial cells of the ovary.......... 356
  Historical 356
  Present investigation 365
    Material and methods.. 365
    Results 367
      Condition during autumn.. 367
      In the winter during hibernation.. 369
      After hibernation, during rutting.. 371
      Captivity and isolation during spring.. 375
      During pregnancy and lactation.. 376
      In midsummer 379
    Conclusions 381
Origin of the interstitial cells of the ovary.. 381
Restudy of the interstitial cells of the testis.. 387
  Presence and behavior of mitochondria.. 387
  The source of the pigment granules.. 390
Comparison of interstitial cells of the testis and ovary....... 391
Summary 394
Bibliography 396

INTRODUCTION

The significance of the so-called interstitial cells found in the ovary and testis of many mammals and of some lower orders is still a much-debated subject. Since the introduction of the term "interstitial gland" by M. Bouin in 1900 with reference to the ovary and later applied to the testis by P. Bouin and P. Ancel ('03), these cells have received much attention in connection with the supposed internal secretory activity of the gonads, for which there is much evidence not only from experimental but also from strictly clinical sources and especially in the rapidly increasing field of organotherapy. One is particularly struck with the number of reports in current clinical literature on the more or less successful use of ovarian extracts and transplants in female disorders.

The question of cyclic changes in the interstitial cells of the testis and the relation of such periodicity to the various sexual phenomena, has been recently considered (Rasmussen '17) in a paper on the occurrence of a definite and well-marked seasonal hypertrophy of these elements in the male woodchuck. While similar changes have been reported in other animals, the various phases of atrophy and hypertrophy do not correlate well with any particular one of the other sexual phenomena. The interstitial cells of the testis are taken up again in this paper because a number of cytological details have been reinvestigated and because a comparison of the cyclic changes in these elements and the homologous cells of the ovary seemed desirable.

Essentially the same problems that are met with in the testis are encountered in the ovary, where similarly there are several tissues to which the endócrine function may be attributed and where there is much uncertainty as to the exact role played by each of the morphological components.

Of the many lines of inquiry followed in working with the interstitial cells of the ovary, a number stand out rather prominently; namely, comparative investigation as to their presence and relative abudance in the adult of different species, their morphological alteration during various periods of life as correlated with the sexual cycle, the effects of ovarian extirpation and transplantation, and their origin. The effects of X-rays and of ovarian extracts together with histological findings in the ovary in various functional and developmental disorders, have also yielded many suggestive facts. It is not the purpose here to analyze the mass of literature that has necessarily accumulated since this has been done at various intervals and from various points of view in many papers of which the more recent ones are those by Anna Schaeffer ('11), Van der Stricht ('12), Pardi ('14a, '14b), Aschner ('14), Kingsbury ('14), Firket ('14) (best review of literature on non-mammalian forms). O'Donoghue ('16) (on marsupials), and Boring and Pearl ('17) (on birds). The publications by Tandler and Grosz ('13), Biedl ('13), Blair Bell ('16) and Falta ('16) may also be mentioned.

In regard to the presence or absence of interstitial cells in the ovary of adult mammals, it may be well to call attention to the fact that of more than one hundred species so far examined—of which Fraenkel ('05) and Anna Schaeffer ('11) alone investigated eighty-one—fully fifty per cent. are said to have none. In many of the species reported as not possessing interstitial cells during adult life, it is known that these elements may be plentiful in earlier stages of development. In some orders, e. g. Ungulata, they are rarely found; while in others, e. g. Rodentia, they are nearly always present. There may be considerable variation in closely related forms. In several cases there is disagreement among workers as to the presence of these cells in the ovary. This variation in opinion is primarily due to a difference as to what constitute interstitial cells and hence an interstitial gland. Falta ('16, p. 370) states explicitly that in women we understand by "interstitial gland" a cell complex that develops from the theca interna of atretic follicles. Many do not, however, agree that these hypertorphied cells are comparable to typical interstitial cells found in the stroma in many other mammalian ovaries.

A second source of error in conclusions drawn from the examination of a few specimens of a given species is the more or less periodic change which the interstitial cells may undergo in animals, so that at certain times they are very inconspicuous and may be largely or even entirely overlooked in preparations stained by ordinary means; while at other times, usually related to active stages in the sexual cycle, they are easily detected. A failure to recognize the possibility of such fluctuations is undoubtedly responsible for some of the disagreement.

The primary object of this paper is to describe such variations in the interstitial cell content of the ovary of an animal, the woodchuck (Marmota monax), which has a single sexual cycle per year, the successive cycles being interrupted by about four months of profound dormancy so that there is no overlapping of any of the phases. The latter point is of much advantage since it is obviously very difficult to make reliable deductions where structures developed in one phase are carried over to the same or some other phase of the following cycle or cycles. The fact that this animal becomes reduced in weight and exists for months without food and with a rectal temperature of 6° C. to 15° C., when normally in the active state the body temperature is in the neighborhood of 37° C., and with all the physiological processes enormously reduced, should also present a situation that might throw some light on the behavior of the interstitial cells under modified functional conditions.

The bearing of these observations upon the problem of the significance of the interstitial cells naturally rests upon the assumption, generally accepted, that functional activity is correlated with structural changes, although it has not been proved that hypertrophy of these cells corresponds to increased secretory activity upon their part. One must agree with Gley ('17, p. 212) that there is a pressing need for physiological studies testing this assumption.

CYCLIC CHANGES IN THE INTERSTITIAL CELLS OF THE OVARY

HISTORICAL

Because of the availability of material and the size, great abundance and uniform distribution of the interstitial cells of the ovary in the rabbit, this animal has been used more than any other species in investigations of morphological variations in these elements. However the earliest record of such changes is generally attributed to MacLeod ('80) who found that in the mole the lateral and greater portion of the ovary, which represents the medullary portion of the typical mammalian ovary and is separated from the cortical tissue by a distinct groove at certain seasons of the year, is minimal in June. By the last of October the entire ovary has increased to five times its former size and the lateral portion which now constituted nine-tenths of the entire organ was much darker in color and consisted of medullary cords separated by great masses of interstitial cells which were responsible for the enlarging of the ovary. During this increase in the interstitial cells, follicles had been developing and were beginning to undergo atresia. No information is given as to when atrophy takes place. This must occur some time before the following June. While he examined the ermine and bat, no changes in the interstitial cells of these animals are mentioned.

Leydig ('57) noted that the division of the mole ovary into two superimposed parts did not exist at all seasons of the year since he did not find it so in June, but gives no further details; while Wagner ('79), in a description of the modifications going on in the ovary periodically with the recurrence of rut and gestation, considered the medullary portion of the ovary of the mole to be a corpus luteum.

According to Tandler and Grosz ('11), the mole litters during the last of April and first of May—fertilization having taken place in March when spermatozoa are abundant in the male. An increase in the interstitial cells of the ovary thus takes place months after the rutting season. In the testis of the mole of this species, Tandler and Grosz ('11), found a similar hypertrophy of the interstitial cells. This reaches a maximum in August, or about two months ahead of the corresponding changes in the female. In the testis also these elements are minimal during the rutting season.

A suggestion of periodicity in the interstitial cells was made by Paladino ('87), who describes a continual degeneration and regeneration of the ovarian parenchyma (including the interstitial cells) and noted that these processes are not going on at a uniform rate at all ages or even in the adult, being, in the latter case. especially marked at the time of heat and pregnancy, during which times he also noted, especially near the hilus, marked down-growths of the germinal epithelium into the medulla and other evidences of regeneration apparently compensatory for the marked degeneration that had gone on. He worked on the pig, horse, cow, sheep, goat, rabbit, guinea-pig. cat. dog and man and apparently found the above to be true in general.

Child ('97) found the stroma cells of the ovary of pregnant rabbits and of a pregnant dog to be much hypertrophied and to possess a centrosome and sphere—structures not demonstrable by this author in these cells in adult rabbits at other times except shortly after parturition. He could not find a centrosome and sphere in these elements in the cat or in the white rat at any time, although he noted that at the time of pregnancy the stroma cells undergo hypertrophy somewhat the same as in the rabbit and dog. Child is evidently describing changes in what are now generally termed interstitial cells for he found intermediate stages between spindle-shaped stroma cells and polyhedral cells with a spherical nucleus. He states that in non-pregnant rabbits the stroma consists largely of the long spindle-shaped cells with deep staining nuclei and boundaries that are difficult to make out; while the ovaries of pregnant rabbits (and dog) contain relatively few of these spindle-shaped stroma cells, but instead a great many of the larger cells with abundant cytoplasm, one or two distinct centrosome and a vesicular nucleus eccentric in position.

Child raises the question as to what is the relationship of these ovarian changes to pregnancy. A number of subsequent investigators have attempted to throw light on this particular question, using mostly the rabbit as material; yet not only does the question remain unanswered, but there is great diversity of opinion as to what changes if any, in these (interstitial) cells, actually do occur during pregnancy, as the following review of the literature will show.

Lane-Claypon ('06) found in the rabbit an increase in the size of the interstitial cells of the ovary with the progress of pregnancy, reaching a maximum at about the twenty-second day. They increase in diameter from 17 to 32 micra or about five times in volume. The whole ovary increases, which is fully accounted for by the hypertrophy of the interstitial cells. Commencing a few days before parturition, they diminish again, reaching a minimum about six weeks later, which is maintained till the next pregnancy supervenes.

Pardi ('14b) similarly found in the rabbit an increase in size and in lipoid content of the interstitial cells during the major portion of pregnancy, those in the virgin being 22 to 23 micra in diameter while in the pregnant animal they increase to 27 or 28 micra. This increase is solely in the cytoplasm, according to this author. The nucleus remains unchanged. There may also be an increase in number by a transformation of stroma-cells. The whole ovary increases in size directly with these changes.

An increase in the lipoids during pregnancy has been recorded by Ciulla ('09) and Ciaccio ('10). The latter author from a study of the cat, dog, rat and guinea pig, in addition to the rabbit, also thinks that there may be an increase in number of interstitial cells by a hypertrophy of stroma elements.

Others have failed to find any striking change during pregnancy or other phases of the sexual cycle in the rabbit. Limon ('02) maintains that when once formed they remain unchanged. Allen ('04) says that a description of the interstitial cells in a six months old virgin rabbit applies equally to early pregnancy and he is inclined to think from an examination of a number of adult rabbits that pregnancy makes no difference in the number of atretic follicles (from which interstitial cells may be derived). Zalla ('07), who worked on the rabbit as well as on ten other species, could not establish any appreciable or constant change in the interstitial cells during pregnancy. Regaud and Dubreuil ('08), while not denying the possibility of a slight increase during gestation in the rabbit, did not find a regular correlation between degrees of hypertophy of the interstitial cells and duration of pregnancy, all stages of variation in these elements being encountered at any particular stage of pregnancy. They found later ('09) that there is a tendency for this tissue to develop during the spring months.

And finally the third possibility, namely, that the interstitial cells decrease during pregnancy, has been strongly defended for the rabbit, as well as for other animals, by Aschner ('14), who maintains that the interstitial cells of the ovary increase till rut, being maximal at the beginning of pregnancy, and that there is a distinct retrogression as corpora lutea develop. The next period of follicular growth and increased atresia is accompanied by an increase in the interstitial cells.

Montuoro ('03) found a decrease in the amount of lipoids in the interstitial cells during pregnancy in the rabbit; but Pardi ('14b) argues that this must be due to faulty technique, Montuoro having used osmic acid and paraffin imbedding, resulting in a loss of some of the more soluble lipoids.

The rabbit has been used for many other lines of research upon this subject, but the above is believed to be a fairly complete résumé of the particular phase with which we are here concerned. It is evident that in spite of the amount of work done on this supposedly very favorable animal, there is still great need for a careful checking up of this entire line of investigation on this as well as on other species. A number of other animals have received some attention and to the results obtained we may briefly refer.

From an examination of the ovaries of 250 dogs, Aschner ('14) found during pregnancy a similar decrease in the interstitial cell to that described by him in connection with the rabbit. He disagrees radically with Fraenkel ('05, '11), Aimé ('07), Schaeffer ('11) and others who claim that there are no interstitial cells in the ovary of the adult dog. It was noted above, however, that Child ('97) described the ovarian stroma of a pregnant dog as containing polyhedral cells essentially similar to those in the rabbit. While Ciaccio ('10) cites the dog as an example of an animal in which the interstitial gland, represented only by scattered elements, shows marked hypertrophy during pregnancy and moderate infection and intoxication. Aschner explains that the discrepancy in the observations between himself and Fraenkel, etc., may be accounted for on the grounds that these cells are readily changed by disease, inanition, poisons and by defects in the glands of internal secretion. These conditions, he claims, may so decrease the fatty content of the ovary that Sudan III gives no stain in frozen sections. In reviewing the phylogenetic and ontogenetic conditions, he concludes that the higher one goes in the animal scale the more the corpus luteum dominates and the more the interstitial cells are depressed. Such an inverse proportional development of the interstitial gland and corpus luteum was also noted in a number of animals by Cesa-Bianchi ('07a) and Anzilotti ('09), and calls to mind the classification of mammals proposed by Bouin and Ancel ('09), who place in one group animals in which ovulation is spontaneous so that at definite periods there develop one or more corpora lutea regardless of fertilization. Such animals do not have an interstitial gland. In the other group they place animals in which corpora lutea develop only at coitus and hence possess only corpora lutea of pregnancy; but they have an interstitial gland which takes the place of the periodic corpora lutea. Exceptions to this rule have, however, been recorded. This point is discussed by Kingsbury ('14), Loeb ('17) and others and need not be further considered here.

In marsupials O'Donoghue ('16) finds that subsequent to ovulation the interstitial cells and their nuclei undergo hypertrophy, although they never become as large as the cells of corpora lutea. At this time they appear very epithelioid and glandular, and in consequence he regards them as masses of secretory tissue. The maximal enlargement is reached about the time the corpora lutea become fully formed and appear to remain active longer than the luteal cells, being in an active condition long after birth of the young and after the corpora lutea have commenced to decline.

Athias ('16) says that in the guinea pig the interstitial cells increase at puberty and gestation at the expense of atretic follicle. Although Loeb ('17, p. 300) states that just piror to ovulation a sudden simultaneous degeneration of all but the smallest follicles sets in and that the theca interna of atretic follicles is strongly developed five to seven days after ovulation, he regards the ovary of the normal adult guinea pig as not possessing true interstitial cells. In earlier publications ('11, p. 341 and '14, p. 39) he speaks of their being represented only by small shrinking connective tissue cells of the theca interna filling the place of lost parts of the follicles which are in process of atresia. In the most recent paper ('18, p. 48) we find the unqualified statement that typical interstitial cells are not present in the ovary of the normal guinea pig. This divergence of opinion is extreme when we consider that Limon ('02, p. 185) claims that interstitial tissue (composed of interstitial cells) is abundant in the ovary of the adult guinea pig and describes them (p. 165) as being 12 to 15 micra in diameter with a nucleus of 4 to 5 micra in diameter and as forming dense cords and compact masses of cells. Fraenkel ('05, p. 500) says in connection with the four types of ovaries as regards the nature and arrangement of the interstitial cells:

> "Den Haupttypus habe ich in Uebereinstimmung mit Limon oben beschrieben. Der grösste Theil des Ovariums ist von diesem Gewebe eingenommen, welches durch Bindegewebsstränge, die mit Rinde und Hilus communiciren, in Läppchen und Fächer eingetheilt ist. Die Zellen liegen sehr dicht, sind dunkel gekörnt oder enthalten feinste Tröpfchen. Ihn repräsentiren am schönsten die Nager, wie Kaninchen, Meerschwein, die Meerkatze, ferner unter den Beutlern die Petro-und Onychogale."

Bouin and Ancel ('09, p. 465) from their own investigations include the guinea pig with the rabbit, mouse and cat as examples of animals with an interstitial gland in the ovary in contrast with women, primates, dog, mare, sow and cow, which possess no interstitial gland but instead periodic corpora lutea. A number of others have described interstitial cells in the guinea pig ovary specially in connection with cytological studies; but it is not always clear that they have reference to adult animals—the only ones admissible in this discussion.

The technique to be employed in the investigation reported here was tried out on guinea pig ovaries and certainly there are many comparatively large cells (10 micra and more in diameter) with spherical or nearly spherical nucleus (5 micra in diameter) and with lipoid-laden cytoplasm—the three principal criteria of interstitial cells—not only in the theca interna of atretic follicles but also in groups in the stroma between follicles and about blood vessels in the medullary portion of ovaries of an adult animal. It is difficult to see how these can be ruled out from the so-called interstitial cell category. At least—and this is the reason for dwelling upon this point—these are what is now in general meant by interstitial cells and this paper deals with such elements. As for differentiating them from the ordinary stroma elements of the guinea pig ovary, better differentiation was obtained with acetic-osmic-chromic fixer and sodium alizarinsulphonate and crystal violet stain on guinea pig than on wood chuck material. It may still be objected that these groups of cells in the stroma proper are the remains of atretic follicles. O'Donoghue ('16), for example, thinks that during degeneration of follicles some of the theca cells merely pass through a stage in which they resemble interstitial cells, and that the interstitial cells constitute a tissue *sui generis.* To this one can only reply in the words of Kingsbury ('14, p. 73): "So consistently has this mode of thecal origin of the interstitial cells of the adult ovary been supported by the observations of nearly all who have devoted careful study to this phase of the subject that it may be accepted as clearly proven." As to whether these cells collectively constitute a gland is a separate question. One must agree with Loeb ('17) and many others that there is a lack of clearness in the definition of interstitial gland of the ovary, to say nothing of its specific function.

Since a large number of investigators considers that the hypertrophied cells of the theca interna of atretic follicles in women are homologous to the interstitial cells of the stroma in animals, a consideration of the human ovary must be included in this review if it is to be reasonably complete; for an increase in atresia and the accompanying hypertrophy of the theca interna cells of involved follicles, has been described in the human ovary during pregnancy by De Sinety ('77), Cohn ('03, '09), Böshagen ('04), Pinto ('05), Seitz ('06), Basso ('06), Walwart ('07, '08), Fellner ('09), Greggio ('10), Pottet ('10), Wolz ('12), Meyer ('13), Keller ('13), Pardi ('14b) and Schottländer ('14). While all these authors do not agree as to the significance of this hypertrophy, most of them consider it an expression of secretory activity. Wallart and Schottländer maintain that here is a similar increase during menstruation in human subjects. Fraenkel ('05, '11), Aimé ('07), Bayer ('08), Bouin and Ancel ('09), Schaeffer ('11), Foster ('17) and others can, however, see nothing in the adult human ovary that could be considered an interstitial gland, although Fraenkel ('05) describes an enlargement of the theca cells during pregnancy. Aschner ('14) from an examination of seventy-eight cases concluded that the interstitial cells are not only much less in evidence in the adult and even at puberty than at birth, but decrease still farther with the appearance of corpora lutea—a general principle that this author finds applicable to a number of different animals as already noted. He considers, however, that the interstitial tissue constitutes a distinct morphological structure.

The whole subject is fraught with uncertainty and conflicting opinion. This is probably illustrated nowhere else so well as in the case of the human subject. To illustrate this point, although not strictly pertaining to cyclic changes a number of recent opinions may not be out of place. Thus Kingsbury ('14), while finding no difficulty in demonstrating interstitial cells in the human ovary (presumably adult), regards them as transitory elements coming from and returning to ordinary stroma cells, and finds it difficult to escape from the conclusions of Fraenkel ('05, '11) that a tissue so inconstant in its presence, even in mammals, does not deserve the dignity of being termed a gland. This is the opinion also of O'Donoghue ('16). Blair Bell ('16) greatly minimizes the importance of the interstitial cells, claiming that the gonads as a whole play only a subservient part in connection with a determination of secondary sexual characters and other internal secretory functions ascribed by many to this organ and in particular to the interstitial tissue. Falta ('16), on the other hand, says that the function of the interstitial gland in man is today already so sharply delimited and is concerned with functions so important and extensive in influencing the conformations of the body, that it is hard to believe that so important an organ is absent in women.

In contrast to the uncertainty in regard to cyclic changes in the interstitial cells of the ovary of many of the forms so far discussed, is the marked annual periodicity described in a number of hibernating animals.

Cesa-Bianchi ('07b), from an examination of serial sections of more than one hundred ovaries of the bat, found that in late March and early April, immediately after the hibernating period, the ovary increases to several times its former size, due to a great increase in interstitial cells, which become completely filled with lipoid and pigment.

In May and June when nearly all females contain foetuses, the ovary is still large and yellow but follicles are so reduced in number that one would hardly recognize the organ as an ovary. The interstitial cell masses have become lobulated rather than columnar. This condition prevails during the remainder of the summer.

Just before bad weather sets in in the autumn and before dormancy intervenes, the interstitial cells rapidly decrease and finally well into the winter season have nearly disappeared. The ovary loses its yellow color and diminishes in volume.

It is to be remembered that in the bat copulation takes place in the autumn; but fertilization and segmentation of the ovum does not occur until after waking up in the spring, when hypertrophy of the interstitial cells again occurs.

Similar changes, except to a less degree, were observed by this author in the badger, squirrel, marmot and hedgehog.

Van der Stricht ('12) noted an increase in the number and size of interstitial cells of the ovary in the bat during winter and particularly in the spring after waking up. The nucleus of these cells becomes slightly larger and stains less densely. The cells become arranged in acini-like groups which are especially numerous and conspicuous during second maturation and ovulation and persists during the summer to a lesser and lesser degree as autumn is approached. In September and October such groups are no longer in evidence, the interstitial cells having materially decreased.

Aschner ('14) states that a decrease in the fatty content and the interstitial cells of the ovary of the hedgehog and tenrec (Centetes ecaudatus) takes place during hibernation, while an increase occurs in the spring. This investigator suggests that the large amount of interstitial tissue in the ovaries of rodents, insectivores, bats, etc., may be related to the fertility of these animals, since a great many follicles are produced and hence much atresia follows. Paladino ('87) similarly noted that degeneration and regeneration in the ovary is more extensive in the more prolific animals.

Only two references were found which dealt with the interstitial cells of the ovary of a hibernating animal of this country. In connection with the subject of hibernation and the pituitary body, Cushing and Goetsch ('15) incidentally report that in the ovaries of two hibernating woodchucks killed in February, there was a small amount of interstitial tissue, but no interstitial cells were definitely recognized. There were no mature follicles. In an animal sacrificed late in March, one ovary contained several corpora lutea and but little interstitial substance containing a few immature follicles. In a fourth animal, which was sacrificed early in May and which had been in captivity all winter and awake several weeks, there were fully formed follicles and one corpus luteum and an increase in the interstitial cells.

Drips ('17), while studying primarily the corpus luteum of the spermophile (striped gopher, thirteen-lined ground-squirrel or technically Citellus tridecemlineatus), noted that the interstitial cells were most conspicuous during early pregnancy and least noticeable just before hibernation took place.

On the whole it appears that an observable increase in the size and possibly in the number of interstitial cells of the ovary of some animals occurs more or less synchronously with rutting and may persist to some extent during pregnancy. There is, however, so much disagreement in connection with several of the species studied that it is impossible to draw conclusions as to the extent of such changes. It is evident that a serious obstacle in the way of accuracy in judging of the degree of hypertrophy or atrophy in the interstitial tissue in most animals, is the irregular and scattered arrangement of the cells. Even in the rabbit, where they are most regularly arranged, there are great differences in the recorded observations. There is, in consequence, a necessity for much detailed cytological work along this line before it is clear just how extensive cyclic changes occur and with which phases of the general sexual cycle such alterations are related.

PRESENT INVESTIGATION

Material and Methods

This report is the result of an investigation of 144 (72 pairs) of ovaries taken from woodchucks killed at various seasons of the year, the technique employed being the same as that used on the interstitial cells of the testis (Rasmussen '17).

All the animals except two were captured in the vicinity of Ithaca, New York. The ovaries of the two exceptions were obtained from Dr. T. G. Lee of the University of Minnesota. They had been collected somewhere south of Minneapolis several years ago and were well fixed in Zenker's fluid and preserved in alcohol. I take this opportunity to thank him for this material which was especially valuable because both the animals from which it was obtained were pregnant, one containing six very young foetuses (uterine enlargements being 8 mm. to 9 mm. in diameter) and the other one seven older foetuses 40 mm. to 45 mm. in length.

The 72 animals involved were killed at intervals so that a larger number were sacrificed at the more critical periods. The number killed in the respective months of the year are as follows: 1 in January, 7 in February, 6 in March, 12 in April, 9 in May, 1 in June, 13 in July, 10 in August, 1 in September, 4 in October, 2 in November and 6 in December. While most of those used between the months of October and the following March had necessarily been kept in captivity from the preceding September, a few controls were obtained directly from the normal habitat in February and March. This is obviously important because these animals do not breed in captivity, as far as our experience goes, and unless material is available from animals that have not been kept for any length of time in captivity, one would not be justified in concluding that the findings represent the conditions as they exist normally. All the illustrations on the ovary accompanying this paper except figures 8 and 17 are from animals killed either in the field, immediately upon being brought to the laboratory or at most two days after being captured. After March and until October material was available again directly from the normal habitat.

The artificial burrows in which nearly all the animals were allowed to hibernate in captivity have been previously described (Rasmussen '15). These burrows are such that the conditions are essentially the same as in the natural ones and in them woodchucks have successfully become dormant for a number of years. Five animals involved were shipped to the Institute of Anatomy, University of Minnesota, where they were kept in a small room in which the temperature was kept between —5° C. and +13° C. Only occasionally and then for only a few hours did the temperature go below 0° C. and above +10° C. during the hibernating period.

In addition to the technique used on the testis as referred to above, fifteen animals were injected with neutral formalin and potassium dichromate as advised by Cowdry ('16b) and quoted by Scott ('16). Perfusing the animal with Locke's solution sufficiently to produce a degree of edema in the ovaries before the fixer is allowed to enter the vascular system was found especially useful in opening out the dense stroma and separating the individual cells, making them stand out more prominently. This material as well as that fixed with the aceto-osmic-chromic fixer used on the testis, when stained with acid fuchsin and methylgreen according to the directions given by Cowdry, gave the most instructive cytological picture by bringing out in sharp contrast mitochondria and other granules in the cytoplasm. If sections from material fixed in solutions containing osmic acid are passed rapidly through the higher alcohols and clearers, covered with a thin layer of xylol-balsam and using no cover glass, but allowing the balsam to dry quickly, the lipoid granules darkened with the osmic acid may be retained. If very much lipoid is present, other cytoplasmic granules are masked too much and it is then better to allow the large lipoid granules to be dissolved out. In this case a cover is used. The lipoid then appears as vacuoles. It will be interesting to see how the acid fuchsin and methylgreen stain will last in uncovered preparations since it is known to fade in a year or two ordinarily when covered.

While in most cases it was necessary to cut both ovaries into pieces to provide material for the various fixers, a sufficient number of whole ovaries were fixed to provide for complete serial sections representative of the different seasons of the years. Sections were cut 3, 5 and 10 micra in thickness. For cytological details the thinnest sections were necessary.

Results

Conditions during autumn.—From September to November the ovaries are small, each varying from .0005 to .0015 per cent. of the net body weight. During this period there is a slight increase in size as winter is approached, due largely to growth of follicles. There are no corpora lutea present. Only occasionally is there any evidence of such structures having been present, the evidence being the presence of large pigmented cells in an area composed mostly of connective tissue still more or less radially arranged. Follicles in nearly all stages of development are present (Fig. 1). Since both ovaries are alike in practically every case during this and other periods of the year when no corpora lutea are present, the percentage weights given refer in all cases to a single ovary.

In the stroma both of the cortex and medulla are many cells varying in size from 4 to 13 micra in average diameter (most of them being from 5 to 9 micra), containing a round vesicular nucleus from 3.8 to 7 micra (mostly 5 micra) in diameter, with a variable amount of cytoplasm as shown in figure 24. The cytoplasm is rarely free from one or more comparatively large lipoid granules that darken with osmic acid. These are the so-called interstitial cells as distinguished from the ordinary stroma cells which have an elongated more or less spindle-shaped granular nucleus and only a small tip of cytoplasm (containing a few mitochondria) at each end.

The variation in size and shape of the interstitial cells and the lipoid (shown in black or as vacuoles) and mitochondrial content (red) are shown in figure 24. The size of the cells varies directly with the lipoid content. There are only a few cells with as much lipoid as is shown in cell e of this figure. As a result it is difficult to give an adequate idea of the amount of interstitial tissue in a general view. In figure 1, which is a photograph of a longitudinal section through an ovary of this period, the darker areas are due to the osmic acid of the fixer having blackened the lipoid of the interstitial cells. The amount of lipoid in this particular case is, however, greater than is usually the case at this time of the year. In many instances a photograph of this kind would show practically no dark areas in the stroma, but appear essentially as figure 7, not because there are no interstitial cells but because each cell contains only a few lipoid granules and many sections through cells show none, as in cell b of figure 24, and hence there is not enough contrast to show anything at so low a magnification. (X 7½).

In the cortical portion of the ovary there are usually masses of interstitial cells closely associated with the follicles, occupying a triangular area on the hilus side of the follicle. Irregular rows and columns of cells are also found in regions between follicles. These have their long axis directed towards the hilum. The septa of ordinary stroma and the blood vessels undoubtedly are responsible for this arrangement. In the more central part of the ovary, the interstitial cells are in close proximity to the blood vessels and medullary cords, often forming a wall about these structures. This relationship to the vascular system and medullary cords is shown in figure 10, which, however, is a photograph of a later stage when the interstitial cells have much enlarged. The interstitial cells constitute the dark areas. In the center of many of the interstitial cell masses is a medullary cord, not seen very plainly in the photograph. The close association of interstitial cells with the blood and lymph vessels has been described by many authors. Some have taken this as evidence of the secretory activity of these cells.

Mitochondria are packed closely in the scanty protoplasm about the nucleus and between the large lipoid granules. Nearly all mitochondria are spherical as may be seen in figure 24. In a preliminary report of this work (Rasmussen '18) it was stated that there were also short rods. It appears from a closer study of the best fixed material that these rods can usually be resolved into two granules. Still there are a few very short ones, from 1½ to 2 times as long as they are wide, each of which appears to be a single body.

Mitochondria have been described in the interstitial cells of a number of animals by Mulon ('10), Athias ('11, '12), Levi ('13), Cattaneo, and Pardi ('14b), usually as rods and granules. Padri in the rabbit indicates only 6 to 8 rods to 100 or 200 round mitochondria.

In the woodchuck the mitochondrial content appears to vary directly with the size of the cells during this stage.

The nucleus is usually somewhat eccentric, nearly spherical, with a very attenuated chromatin network and one or two distinct nucleoli. There are some elongated cells, as figure 24a, in which the nucleus is also elongated. These are evidently transitional stages in the formation of typical interstitial cells from the ordinary stroma cells or cells that appear to have migrated from the germinal epithelium as will be discussed later. In these elongated cells the chromatin is more granular (not indicated in the figure because in the over fixation necessary to bring out mitochondria, the nucleus appears nearly homogenous with the acid fuchsin-methylgreen stain. The nucleus tends to vary in size directly with the size of the cell. In general this is also true of the nucleolus.

In winter during hibernation.—Hibernation commences in November and continues till some time in March. During this period, just as in the autumn, the ovary shows great individual variations in weight, varying from .0008 to .0023 per cent. of the net body weight. The average weight is about .0014 per cent. This range of variation is met with during all parts of the period, although the ovaries appear to constitute on the average a slightly greater proportion of the body weight toward the end of hibernation. But since during dormancy the body weight falls ¼ to ⅓ of its original weight, this increase does not indicate an actual enlargement of the ovaries. Both ovaries of the same individual are strikingly alike. A section of a typical ovary representative of late hibernation is shown in figure 2. As is clearly evident by comparing this figure with figure 1, there is no follicular development during winter sleep. On the contrary, there is much atresia. Most of the large follicles and many of the smaller ones have become atretic and as usual the cells of the theca interna acquire lipoid and in other respects come to be indistinguishable from interstitial cells in other parts of the stroma. The conditions are the same in animals taken directly from their normal habitat and in those kept in captivity.

Some of the interstitial cells during dormancy are slowly enlarging so that a number are 16 micra in average diameter. A few cells may be even larger, especially late in this period, but most are between 8 and 10 micra. The enlarging is due principally to an increase in the lipoid. As spring is approached there are a greater and greater number of cells containing as much lipoid as is shown in figure 24 e. By the last of February, as shown in figure 2, the interstitial cell masses are noticeably more prominent than before hibernation commenced.

With an increase in the size and lipoid content of the interstitial cells during this period there is an increase in the number of mitochondria, and in the largest cells some of the mitochondria appear somewhat larger while no rods are evident. These large mitochondria may, therefore, only be the elongated ones that have assumed a spherical form. Levi ('13) in the guinea pig and other forms records an increase in the mitochondrial content with hypertrophy of these cells to a certain point and that they persist during fatty infiltration to an extreme degree. Athias ('11, '12), however, in both guinea pig and bats describes the mitochondria as being inversely in proportion to the lipoid, while Pardi ('12b) in the rabbit could see no difference with hypretrophy of the interstitial cells during gestation.

The nucleus has enlarged, being from 4 to 8 micra in diameter (most of them being about 6 micra). The nucleoli tend to enlarge in about the same proportion.

There is therefore no sudden change in the interstitial cells with the onset of hibernation nor any decrease in the activity of these elements during dormancy as far as the criteria of amount of lipoid, number of mitochondria and size and prominence of the cells are concerned. The statements made in the literature that the interstitial cells of the ovary decrease during winter-sleep are in most cases based on the few observations made on hibernating animals in which the period just preceding and the early part of hibernation have not been carefully investigated and where the assumption has been made that since the interstitial cells are more prominent in the summer than in winter, that the decrease has taken place at the onset of hibernation. As we shall show later, in the woodchuck the decrease takes place during full activity in the summer, and as already indicated there is a slow but unmistakable increase in the interstitial tissue during lethargy although no food or water is available for several months. A general idea of the appearance of the stroma at the close of hibernation as compared with that preceding dormancy after a sharp nuclear stain is obtainable by comparing figure 16, which is from an animal killed in autumn, with figure 17, which is from an animal sacrificed March 18 within 48 hours after waking up. In the left hand portion of each figure are many interstitial cell nuclei which appear more or less round in contrast to the long, spindle-shaped nuclei of stroma cells (although there are intermediate forms). The round nuclei are much more prominent and in general somewhat larger in figure 17. These differences are reflections of the differences existing between the entire cells. The irregular distribution of the interstitial cells in the woodchuck makes quantitative statements only of a very general character possible. In the bat it also appears (Van der Stricht '12) that the interstitial cells are minimal during September and October and that they tend to increase during winter.

Aschner ('14), it may be recalled, claims that in dogs the interstitial cells of the ovary are diminished by inanition. The type of inanition involved during hibernation appears not to produce such an effect in the woodchuck, at least.

In the spring after hibernation and during rutting and beginning pregnacy.—Immediately after waking up from winter sleep (which in the vicinity of Ithaca, New York, usually occurs about the middle of March) the rutting season commences and the ovaries enlarge rapidly due to a ripening of numerous follicles and to a rapid increase in size of all of the many interstitial cells that have remained comparatively small. Before the end of March the ovary may represent .0050 per cent. of the net body weight. The lipoids of many of the interstitial cells increase so that the mitochondria, which appear to have reached their limit in number, are crowded in between the large fatty granules as illustrated in figure 25 b, where the lipoid is seen as vacuoles. Others have less lipoid and more cytoplasm rich in mitochondria as illustrated in figure 25 a, where the lipoid granules are represented in black. There are also intermediate forms.

During the early spring increase there appear in many of the larger cells additional granules somewhat larger in size than the mitochondria but having, as far as the technique here employed would indicate, very similar staining reaction. In figure 25, cell a contains three and cell b two such granules. They are best demonstrated in the material fixed in Meves' fluid. They may be of the nature of secretion granlues or they may be other lipoids sufficiently chromated to take the stain. That they are produced from mitochondria, as Mulon ('10) believes to be the case in regard to secretion-like granules in the interstitial cells of the rabbit ovary, is also possible. These granules will be further discussed later.

While females captured in April usually contain foetuses, some may be found not to have become pregnant as late as early in May. In these late cases extreme hypertrophy of the interstitial cells is found. This may be due to a prolonged rutting period, as would be suggested by the results obtained by Regaud and Dubreuil ('09) who found that prolonged cohabitation with the male produced a noticeable hypertrophy of the interstitial cells of the ovary in the rabbit.

The maximal development of the interstitial tissue encountered is shown in figure 3. This is from an animal shot in the field while chasing about with a male (which was also shot and found to possess testes with ripe spermatozoa and interstitial cells maximal) about six weeks after woodchucks were observed in general to become active that particular season. The great size of the ovary is apparent since it is reproduced at the same magnification as figures 1 and 2 and represents .0085% of reduced body weight. Both ovaries were alike. Six ovaries representing this stage were obtained.

The abundance of interstitial cells may be judged from figure 4 which is an enlarged view of the area outlined in white in figure 3. The blackened lipoid granules, which serve to identify the interstitial cells, are here shown with greater contrast. A still higher view of a thinner section with a nuclear stain is shown in figure 18, which is directly comparable with figures 16 and 17. Many of the interstitial cells are over 20 micra in average diameter, containing a nucleus as large as 12 micra and nucleoli as large as 3 micra in diameter. The chromatin becomes more attenuated as the nucleus enlarges. Most of the cells are in the neighborhood of 16 micra and the nuclei about 8 micra in diameter. The size to which these cells may develop is shown in figure 26 b. They are often arranged in irregular groups, the individual cells being poorly separated from each other, as illustrated in figure 26 a. The quantity of lipoid in the individual cell varies from an amount that fills completely the cell as in figure 25 b to relatively a few granules such as is shown in some of the cells in figure 26. The cells not completely filled with lipoid are by far the most numerous in these extreme cases. Such cells contain a correspondingly large number of fuchsinophile granules of various sizes as shown in figure 26. The smallest granules—evidently mitochondria—are greatly masked by the larger ones and are seen only in the best fixed material 3 micra, or less in thickness. The larger granules are more easily fixed and stained, being fairly clear in ovaries fixed in Zenker's fluid and preserved for several years in alcohol before being imbedded and stained with iron hematoxylin. In such preparations they appear essentially the same as the so-called secretion granules of neighboring lutein cells.

Various forms of granules have been observed in the interstitial cells of the ovary of several mammals by a number of investigators by employing copper hematoxylin, iron hematoxylin, and other stains. The fuchsinophile granules figured by Montuoro ('03) in the interstitial cells of the rabbit appear to be very similar to those here described. This author shows cells, containing very little fat, which are practically filled with such granules. He found the interstitial cells of older adults to be relatively richer in metaplasm, possibly due to a replacement of the fuchsinophil granules by fatty droplets such as evidently occurs in the woodchuck a little later when atrophy commences, as will be shortly explained.

In figure 3 it will be noted that there is considerable atresia. In one very large atretic follicle and two adjacent smaller ones the intensely black color is due to the abundance of lipoid in the hypertrophied theca cells. The other smaller atretic follicles scattered throughout the ovary contain less lipoid. There is no essential difference between these hypertrophied theca cells and interstitial cells not related to follicles. The theca cells acquire lipoid more rapidly and tend to become somewhat larger in size. A medium-sized atretic follicle of this period is shown in the center of figure 22. The cytological details of the theca cells may be represented by figure 26. The theca interna (T) can be distinguished from the surrounding stroma (IC) by its heavier lipoid content, indicated in black. There can be no mistaking of derivatives of the granulosa for hypertrophied theca cells in this case because the basement membrane (M) is still intact and within are a few remains of the atrophied granules as well as a fairly well preserved ovum and a little of the discus proligerous indenting the lower side of the egg membrane.

The similarity in appearance of interstitial and lutein cells has been commented on by many writers and especially by Van der Stricht ('12), who found in the bat a marked encroachment of the corpora lutea upon the stroma so that the outlines of the former are not clearly defined, but cords of interstitial cells become continuous with the corpus luteum. Such interstitial cells have the same aspect as lutein cells. There is some similarity between these two elements when the interstitial cells are maximal in the woodchuck. A typical lutein cell is shown in figure 29 and is seen, however, to be much larger than the largest interstitial cells; to be free from lipoids, which blacken with osmic acid; but to be literally filled with granules that resemble in almost every respect the larger fuchsinophile granules of the interstitial cells. In very early corpora lutea there are apparently lutein cells containing a few such fatty droplets but they soon disappear in the case of corpora lutea of pregnancy. Later towards the end of gestation and after parturition, such lipoid appears again, evidently as an expression of degeneration. In corpora lutea resulting from rupture of follicles not followed by pregnancy, the lipoid appears much earlier.

Two such corpora lutea are shown in figure 3 (CL′) where the fatty content appears black.

Usually there is no difficulty in distinguishing corpora lutea from atretic follicles; but in one animal, containing very young foetuses, was found what was taken to be a corpus luteum much younger than the others and with considerable blood in the large central cavity. On following the series along it was found that it contained an ovum (indicated by O in figure 20). The theca interna had already been largely incorporated, as normally occurs in corpora lutea, so that it is not possible to exclude the cells of the theca interna from being the source of the large luteal-appearing cell, especially since what appears to be a few degenating granulosa cells are found about the egg and in the adjacent lutein tissue. The rest of the tissue cannot be distinguished from neighboring corpora lutea of pregnancy. However, only material fixed in Zenker's fluid and stained with iron hematoxylin, was available. In all probability this is another example of an abnormality where the granulosa cells have changed to lutein cells before the ovum has been discharged such as has been described in some other animals (c. f. Corner '15).

Capitvity and isolation during the spring.—Seven females which had hibernated in captivity and after waking had been with active males (which had also hibernated in captivity) for two to four weeks, were sacrificed between April 10 and April 24. None of these animals were pregnant. While the interstitial cells were more prominent than in animals killed just at the close of hibernation, the degree of hypertrophy just described was not found in a single case. Nearly all the interstitial cells, especially in animals that had been awake the longest, were very rich in lipoid and contained many mitrochondria, essentially as represented in figure 25b. They contained, however, very few of the large fuchsinophil granules.

The ovary in no case had enlarged much, if at all. Many atretic and some medium-sized normal follicles were present about as indicated in figure 8. This figure shows the maximum interstitial cell development observed in animals kept in captivity. It is taken from another female which was kept till May 18 (three weeks longer than the seven animals above mentioned) and which had been isolated from males since February 6 preceding. In this animal there had evidently been ovulation, judging from the young corpus luteum present. The other ovary showed two corpora lutea slightly older in appearance. Only two large ripe follicles were encountered in all these eight females kept in captivity during the spring.

Since in the woodchuck captivity does not interrupt the male sexual cycle (as far as the appearance of the testis is concerned, Rasmussen '17), it seems probable that the failure of these animals to breed in confinement is due to its effect on the female. This is suggested by the scarcity of large mature follicles in the ovary. However, M. Regaud, in discussing a paper by Branca ('03) on the testes of certain animals in captivity, reports (p. 198) that in the European marmot (belonging to the same genus as the woodchuck) no spermatogenesis occurs in males kept over winter—the testes in the spring remaining essentially as they are in winter during hibernation at which time the tubules hardly contain any cells of spermatogenic origin.

During pregnancy and lactation.—At the beginning of pregnancy the stroma is thus largely occupied by great masses of enlarged interstitial cells rich in lipoid and other granules. The growth of the corpora lutea together with the fact that frequently there is an unequal number of corpora lutea in the two ovaries, now become prominent factors in the weight of this organ, and are responsible for the unequal size frequently met with from now on till July or August. The ovary with the largest number of corpora lutea is always the heavier, one frequently being several times the weight of the other. The largest ovary obtained was one containing 5 corpora lutea and weighed .285 g. (.01163 per cent of net body weight) and was three times as heavy as the other ovary which contained only one corpus luteum. This was in the last of May, at least several weeks after parturition.

In the initial development of corpora lutea there is an inrush of the theca and neighboring stroma, carrying along with them many interstitial cells. In figure 9 the arrow indicates a group of interstitial cells (identifiable by their lipoid content, shown in black) thus being carried into a young corpus luteum in the direction indicated by the arrow. The fate of these cells cannot be stated. They soon lose the lipoid that blackens with osmic acid and either revert to connective tissue elements associated with the vascular framework of the corpus luteum, disappear entirely or become lutein cells. The source of the lutein cells and the fate of the inrushing theca cells, etc., is still a disputed question and one that cannot be entered into here. Corner ('15), who discusses at length the literature on the subject, believes it probable that in the sow some of the theca cells may remain in the fully formed corpus luteum as special cells distinguishable from the other lutein elements.

With the progress of pregnancy the interstitial cells in all parts of the ovary decrease in size but become relatively richer in lipoid at the expense of the larger fuchsinophile granules as seen in figure 27a, which is a group of two cells undergoing atrophy and in which there are only five of these larger granules left in the particular section drawn. Practically all these coarse granules had disappeared in an animal containing foetuses 45 mm. in length.

Mitochondria, as was also shown by Levi ('13) in a number of other animals, largely persist during this extreme fatty infiltration. The fatty globules of the regressive stages are slightly smaller and more closely packed than those present during the progressive stages and apparently produce a deformity of the nucleus. Occasionally, however, a number of individual droplets may fuse forming spherules even larger than the largest ones represented in figure 26. This lipoid is then gradually absorbed and as a consequence the cells diminish in size, as shown in figure 27, till, in many cases at least, only fragmented and shriveled nuclei are left as in e of this figure. These picnotic nuclei apparently disappear quite rapidly. The interstitial cells left in the stroma between adjacent corpora lutea, are the first to disappear—probably on account of the pressure produced by these growing structures.

By the time of parturition many of the interstitial cells have disappeared entirely and the others are in various stages of decline. A general view of this condition is shown in figure 5. The interstitial cells remaining are in groups which appear intensely black because of the large amount of lipoid. In each dark area will be found side by side all the forms shown in figure 27. This process goes on during lactation. Figure 5 is from a lactating animal in which the uterus still was greatly congested giving evidence of recent parturition.

The figure indicates the large amount of atresia that has taken place. All the follicles large enough to show in the photograph, except one, are in advanced stages of degeneration, each appearing as an intensely black ring indicative of the heavy lipoid infiltration in the theca cells. Normal fully developed follicles may, however, persist for a considerable time after pregnancy has taken place. There were several such follicles in an animal with foetuses 40 mm. to 45 mm. in length.

The particular ovary from which figure 5 was taken contained only two corpora lutea and these were to one side of the median plane so that a section showing a good general view of the stroma nearly missed one corpus luteum and is not through the greatest diameter of the other. However, sufficient is included to show the lipoid which appears in the lutein cells at the expense of the secretion-like granules, as illustrated in detail in figure 31 a.

In passing it may be noted that Van der Stricht ('12) in the bat and Corner ('15) in the pig regard the lipoid that appears in young lutein cells as secretion products. In the spermophile Drips ('17, '18) considers that the fatty droplets which appear in abundance comparatively late in pregnancy, during the decrease in the fuchsinophil granules, and which persist for about six weeks afterwards, as an internal secretion having to do with the normal involution of the uterus. In the woodchuck there seems to be no valid reason for considering the lipoid which appears in the corpus luteum during late pregnancy and immediately thereafter as being different from that which two months later completely fills these cells (fig. 6) and which evidently is an expression of degeneration. The uterus in the woodchuck appears to return to normal rapidly and long before there is very much lipoid in the lutein elements.

The gestation and lactation periods are apparently not known in the case of the woodchuck. The facts could not be determined because the animals would not breed in captivity. General observations show, however, that they become permanently awake during the second half of March (in the locality concerned) and most of them soon become pregnant for the majority of those caught in April contain foetuses. Lactating animals are obtainable in May and many young are seen in June.

In midsummer.—By July the interstitial cells have greatly diminished. In the last half of the month the medullary portion of the ovary is very poor in lipoid (fig. 6). There are, however, many cells nearly round in form and from 4 to 8 micra in diameter (nucleus from 3.8 to 6 micra), which rarely contain more than two or three lipoid granules and many that contain none. Such cells are fairly rich in mitochondria, considering the scanty cytoplasm. They may be represented by b and d of figure 24, d of figure 27 and figure 28. In the cortical zone there are in addition a considerable number of cells with somewhat more lipoid and occasionally as large as 12 micra. There are also many cells entirely free from lipoid with more or less elongated nuclei as well as spherical which appear to have been newly derived from cells that have migrated down from the germinal epithelium as shown in figure 21 and as will be discussed under another heading. It is therefore difficult to say how many of the interstitial cells of this stage are survivals of the retrogression of the hypertrophied cells of earlier months and how many are new derivatives from either ordinary stroma cells or other cells which apparently have migrated into the stroma from the germinal epithelium and which migrate into the masses of atrophic interstitial cells and degenerating corpora lutea. The appearance of the center of the larger groups of retrograding cells into which no migratory cells appear to have penetrated, would indicate that many interstitial cells persist as lipoid free cells essentially like figure 24 b. The nuclei of other stromal elements that transform into interstitial cells do not become round before more or less lipoid has made its appearance in the cytoplasm.

Now and then a cell, such as is shown in figure 28, is encountered which almost certainly is derived from the larger lipoid-laden cells, as is evident from the presence of a large spherule which appears to be the remains (now probably pigmentary in character and more resistant to absorption) of the granular content once so abundant. This is altogether probable for pigmentary degeneration of the hypertrophied interstitial cells, while not common such as it is in the testis of this species, does occasionally occur, and a few large pigment-laden cells are seen at this time of the year and later. Such cells have all the characteristics of the pigment cells of the testis which will be mentioned again a little later. They are easily picked out by copper hematoxylin, iron hematoxylin, acid fuchsin, crystal violet and Sudan III after chromation because of the lipochrome content.

The lutein cells have by this time become densely infiltrated with lipoid so that the corpora lutea, which may still be maximal in size, make exceedingly conspicuous structures in material fixed in osmic acid, as is illustrated in figure 6. Most of the lutein cells in such corpora lutea are like figure 31 b, although all the cells are not in the same stage of retrogression. After this stage has been reached, the lutein cells rapidly lose their lipoid and finally disappear completely. Figure 31 shows several stages of this fatty infiltration and the subsequent atrophy.

In August, corpora lutea in various stages of atrophy are regularly seen. With the disappearance of the corpora lutea, the ovary diminishes greatly in size, falling down to about .0008 per cent of the body weight. A typical ovary just before the last traces of corpora lutea have been absorbed is shown in figure 7. The black area consists of atrophied lutein cells that have not yet disappeared. In such degenerating corpora lutea are found a few cells, such as are shown in figure 31 d, resembling interstitial cells. Their source is not clear. They may have been derived from lutein elements as Van der Stricht ('12) suggests. This investigator asks the question if it might not be that such cells as appear not to degenerate completely in the bat are the original theca cells. It has also been mentioned that there is a migration of cells into such degenerating corpora lutea from the outside and this may be the source of the cells in question. Certainly the lutein cells practically all undergo complete atrophy and leave very little evidence, aside from an occasional pigment cell, of their former presence.

There is during this period and also earlier, during late gestation, renewed development of follicles. Primary follicles become very numerous and some of these are enlarging and sinking deeper into the ovary. Mann ('16) and Drips ('17) describe a similar development of follicles before the period of hibernation in the spermophile.

Conclusions

The observations seem to warrant the conclusion that there is an annual cycle going on in the ovary of the woodchuck in connection with interstitial cells. This consists of a very slow hypertrophy of some of the cells during late autumn and during hibernation, which is followed by a more rapid development, in which all the interstitial cells are involved, during the spring after waking up from winter-sleep. The maximum is reached at the time of ovulation and beginning pregnancy and especially in those individuals that do not become pregnant till a month or so after the majority of the females conceive. Retrogression consisting of a decrease in size and number of cells, follows with the progress of pregnancy and by July, which is several weeks after lactation has ceased, they have reached a minimum. During retrogression there is renewed activity in the downgrowth of cells from the germinal epithelium which appears, as will now be discussed in further detail, to give rise to new elements that transform into interstitial cells which, in connection with those that have resulted from atrophy of the enlarged cells of the existing cycle, are ready to commence the next cycle.

ORIGIN OF THE INTERSTITIAL CELLS OF THE OVARY

The origin of the interstitial cells of the ovary has been much debated. His ('65), Tourneux ('79), Janosik ('85, '88), Child ('97), Plato ('97), Coert ('98), Rabl ('98), Regaud et Policard ('01), Limon ('02), Allen ('04), Saimont ('05), Aime ('07), Von Winiwarter ('08), Ciaccio ('10), Popoff ('11) (in the dog), Athias ('11), Van der Stricht ('12), Kingsbury ('14) and Firket ('14) all have derived them directly from the stroma cells, which are considered a type of connective tissue. O'Donoghue ('16) admits that in marsupials they may have arisen from the stroma cells at a very early stage. Janosik ('88), Schottländer ('91, '93), von Kölliker ('98), Rabl ('98), Clark ('98), Regaud et Policard ('01), Van der Stricht ('01, '12), Limon ('02), Cohn ('03, '09), Allen ('04), Saimont ('05), Seitz ('06), Aimé ('07), Wallart ('07), Regaud et Dubreuil ('07, '09), Benthin ('11), Popoff ('11) (in weasel and mole), Aschner ('14) and Kingsbury ('14), that is, many of the authors already named and some others, consider that they also come from the cells of the theca interna of atretic follicles. This would appear, after all, to be only an indirect derivation from the stroma cells and one that is prominent in later stages of development and in the adult. v.d.Broek ('10) insists that in marsupials they originate independent of either atretic follicles or corpora lutea. Against the connective tissue origin are the views of Nussbaum ('80), Schulin ('81), Horz ('83), Paladino ('87), Lane-Claypon ('06), Ganfini ('07, '08) and M'Ilroy ('17), who believe they are epithelial in origin, being derived from the mesothelial covering of the ovary. Popoff ('11) believes there is a double origin in the mole where they appear to originate also directly from the epithelium as well as from the theca interna. Firket ('14) describes also a double origin in the chick where the interstitial cells appear to come both from the stroma and from the medullary cords. But since in the fowl ovary some of the elements that have been considered interstitial cells by many are now regarded by Pearl and Boring ('18) as lutein cells which appear in groups mostly in the theca interna, it is not clear how this will affect the conclusions of Firket.

A number of other sources that have been suggested, especially by older workers, are of historical interest only and need not be mentioned here.

It appears, therefore, that of the two sources of interstitial cells, the stromal origin, either direct or indirect through the theca interna of atretic follicles, is held by the vast majority—the epithelial as the exclusive source or in addition to the stromal origin being supported by only a small minority.

A thorough-going investigation into the embryology has been carried out on so few animals that it is not surprising some disagreement should prevail and possibly there is some difference in animals of different species. Then too, the ordinary stroma cells of the ovary are not all typical connective tissue and if traced far enough back it is difficult to exclude them from having arisen from the coelomic mesothelium. There is some typical connective tissue, especially along the larger blood vessels and in the tunica albuginea. This in general has migrated into the ovary through the hilum. It appears altogether probable that many of the stroma cells may retain, even in the adult, many embryological characteristics so that they are capable of further differentiation such as occurs during ripening of follicles and atresia when the theca cells become quite different from the ordinary stroma cells, from which they are almost universally recognized as having been derived. If such hypertrophy can take place, there should be no serious difficulty in understanding the derivation of interstitial cells from ordinary stroma cells not related to follicles. In mammals the hypertrophied cells of the theca interna, especially of atretic follicles, are regarded by the great majority of investigators as differing in no essential from other interstitial cells—a view also fully supported here upon the evidence presented by the woodchuck (fig. 22). Such a connective tissue (stromal) origin has, however, been urged as an argument against the theory that they are secretory in function.

This subject of the origin of the interstitial cells of the ovary, while in a sense quite separate from that of cyclic changes, was forced into the discussion because of the activity noted in the germinal epithelium in adult woodchucks especially during the rapid decline of the interstitial cells. Even during the latter part of dormancy the epithelium shows some degree of activity judging from a thickening seen here and there due to a prolifieration of cells so that a second layer forms beneath the superficial one, as is illustrated at point a in figure 21. Such activity increases with awakening when cells soon appear to be leaving the germinal epithelium, penetrating the tunica albuginea usually in a very oblique manner, as illustrated in figure 11. During pregnancy this migration is conspicuous. A general view from a pregnant animal (foetuses 40 to 45 mm. in length, killed April 17) is shown in figure 11. A more detailed view of the superficial part of the same section is shown in figure 12. The cells of the germinal epithelium are seen to form a very irregular outline in places. The degree of differentiation of the stain (iron hematoxylin) has left the nucleus of the cells black while the outlines of the cytoplasm are indistinguishable. The ordinary connective tissue cells of the tunica albuginea are not visible. This contrast is possible because of the larger size and hyperchromatic character of the nuclei of the cells of the germinal epithelium and of those cells that appear to migrate from it.

These migratory cells are apparently somewhat amoeboid and in penetrating the tunica albuginea assume very irregular shapes as is recognizable in figure 13 and shown in more detail in figure 23 (d, e, f, g, h, i). In most places the tunica albuginea remains intact, making it necessary for the cells to take a very oblique and devious course in order to get through as shown in figures 12 and 13. In a few limited areas, as shown in figure 14, the tunica albuginea becomes interrupted due, apparently, to the large number of cells passing through it. In such places the cells stretch in a continuous mass from the germinal epithelium to the cortical layer of follicles.

In penetrating into the ovary some of these cells surround and come in close association with both primary and secondary follicles as shown in figure 15, where they may be recognized by the dark oblong nucleus. They are seen frequently to be in contact with the basement membrane of the granulosa and in all probability may become constituent parts of the theca interna. Deeper in the ovary they enter groups of interstitial cells and since there exist all the intermediate forms that one could wish, as illustrated in figure 19, between them and typical lipoid-laden interstitial cells with vesicular nucleus, it appears that they are a source of interstitial cells.

Wherever these cells are encountered, some of them give the appearance at first sight of being in process of division. The rare occurence of any form of multiplication in the interstitial cells of adult animals, stimulated a more careful study of such apparently dividing cells. A variety of representative cells are shown in figure 23 (d, e, f, g, h, i). It seems that what appeared to be a typical chromosome arrangement in dividing cells as suggested in f and g of figure 23, is really due to folding of the cells, possibly because of the resistance offered by the stroma. Although there are many suggestions of amitotic cell division, such as is shown in figure 23 e, no unmistakable evidence that such actually occurs was seen. Direct cell division has however been described in the interstitial cells of the ovary. Some of the figures published by Regaud and Dubreuil ('06) as evidence of amitosis in young interstitial cells of the rabbit ovary simulate closely what has just been described here in the woodchuck.

One disappointing feature is the scarcity of valid proof of cell division in the germinal epithelium such as would be expected if the cells are rapidly increasing in number. Only occasionally is a mitotic figure seen (fig. 14 M). Such forms as are shown in a and b of figures 23 are numerous, but these are only sharply bent cells evidently due to lateral pressure in the epithelium. A possible factor entering here is the fact that nearly all ovaries after removal from the body were weighed on an ordinary chemical balance and unprotected from the drying influence of the air. As a consequence the small organ rapidly cooled and the germinal epithelium, except in protected places, was affected by drying. However, a few ovaries were dropped immediately into the fixer and still in these evidence of much cell division is wanting.

Later in the season, in July and August, when the corpora lutea are rapidly degenerating and the interstitial cells are greatly reduced, groups and columns of cells from the germinal epithelium penetrate the tunica albuginea in regions between corpora lutea and near the hilum, as is shown in figure 21. Some of these groups, consisting of one cell surrounded by a few slightly smaller ones, are suggestive of oögenesis. The formation of definitive ova in nearly mature animals by a differentiation of cells from the germinal epithelium, has been described by von Winiwarter and Sainmont ('08) in the cat and by Kingery ('17) in the white mouse, showing that the tunica albuginea is no barrier to a migration of groups of cells from the epithelium to the interior of the ovary, at least in some animals, and that oögenesis may take place long after it is generally supposed that such occurs, as far as participation of the germinal epithelium is concerned. In the woodchuck, however, there are not the necessary intermediate stages between such groups of cells as shown in figure 21 b and primary follicles to warrant the conclusion that any of these cells leaving the germinal epithelium in groups in adult woodchucks ever become oöcytes. It appears rather that they form cords that extend into the medulla or remain in the more superficial part of the ovary as irregular masses and cords of cells as illustrated in figure 21 in the region about the point marked C.

The nucleus of these cells when first formed tends to double on itself. Not rarely is a picture such as is represented in figure 23 c observed where every cell in a cross section of one of these cords has a curved or s-shaped nucleus.

The cell cords and especially those that extend into the medullary region become separated from the surrounding stroma by a well defined membrane. Such walled-off cells do not appear to change much the year around. At least during hypertrophy of the interstitial cells there are many unaltered medullary cords and no evidence that any have changed to interstitial cells. It appears quite different with the great bulk of the cells that remain in the cortical zone and do not become organized into definite cords. Transitional forms between these and typical interstitial cells are seen in these, especially during spring hypertrophy, but even late in summer and in autumn many of them acquire a few lipold granules and appear in every way to become interstitial cells which must in a large measure take the place of those that disappear annually.

Downgrowth of the epithelial covering of the ovary of adult animals has been described in a number of animals. Paladino ('87, '88) noted that in the adult pig, horse, cow, sheep, goat, guinea pig and woman, the tunica albuginea does not prevent epithelial invaginations from occurring. He believed that such downgrowths replace degenerated structures and found that in old age the process tends to be limited to the region near the hilum, although it may take place anywhere. Regaud and Policard ('01) show tubular or columnar masses of cells entering the ovary from the epithelium of adult dogs, and emphasize the secretory character of the constitutent cells, having demonstrated what they consider to be secretion granules. The hyperchromatic character of the nucleus of some of the cells is also considered by these investigators to be related to secretory activity. Such diverticula are not present in all species. There are frequently crevices and fairly deep depressions in the ovary; but these do not penetrate the tunica albuginea and are not to be confused with the downgrowth described above.

While it is thus believed that the germinal epithelium contributes to the formation of interstitial cells of the ovary of the adult woodchuck, it is also equally certain that the ordinary stroma cells appear to hypertrophy and become interstitial cells. During rapid development of the interstitial cells, long spindle-shaped stroma cells are seen which are much richer in mitochondria than usual (fig. 30, a). Others are found with a larger, more nearly round and less granular nucleus and still more mitochondria and usually a few lipoid droplets as figure 24, a, and figure 30, b. There are also abundant forms all the way to typical interstitial cells (fig. 19).

The indications are, therefore, that the interstitial cells which disappear annually are replaced by cells from the germinal epithelium as well as from the stroma directly and also indirectly through the theca interna. Nothing can be said here about the origin of the stroma since no embryological work has been done on this species.

RESTUDY OF THE INTERSTITIAL CELLS OF THE TESTIS

THE PRESENCE AND BEHAVIOR OF MITOCHONDRIA

In the recent report on seasonal changes in the interstitial cells of the testis of the woodchuck already referred to, it was shown that the ordinary interstitial cells, containing only a slight amount of pigment and occasionally none at all and shown in figure 32, a, b, c, are minimal in late summer and autumn and remain so during hibernation. With awakening in the spring there follows an increase in the size (fig. 33, a) and number, finally becoming many times their former size (fig. 34, a) and remaining hypertrophied till midsummer when most of them decrease (fig. 35, a) and soon return to their original minimal size (fig. 36, a, b, c). A number, however, were described as not decreasing materially in size but as undergoing a form of pigmentary degeneration, resulting in the formation of cells literally filled with a yellowish lipochrome in the form of granules of various sizes (fig. 36, p) and which are very resistant to fat solvents. These pigment cells are fairly conspicuous in autumn and during hibernation (fig. 32, p); but decreases in size and in number more rapidly during the spring (fig. 33, p and fig. 34, p) and almost or entirely disappear during general interstitial cell hypertrophy.

It was further stated that with the technique employed all attempts to bring out mitochondria in the two types of interstitial cells of the testis, failed and that this point would need further investigation. Whitehead ('12) finally described in the interstitial cells of the pig testis what must evidently be regarded as mitochondria. Van Winiwarter ('12) has demonstrated their presence in the form of granules and filaments in all the interstitial cells of the human testis, but in less quantity where other cytoplasmic inclusions are more numerous. In the opossum they were observed by Jordan ('11) and have recently been described by Duesberg ('18) as being exceedingly numerous, mostly rods, short and curved, and crowded into several heaps. Since these structures are so generally present in other living cells, it seemed improbable that they should be absent in the interstitial cells of the woodchuck testis.

Having had no difficulty in demonstrating mitochondria in the homologous cells of the ovary and in other tissues of the woodchuck especially with acid fuchsin and methyl green after either neutral formalin-bichromate or acetic-osmic-chromic fixation, 14 additional animals were injected with neutral formalin and potassium bichromate as advocated by Cowdry ('16b). This new material and that part of the tissue from the former study which was well fixed in the acetic-osmic-chromic mixture were investigated principally by staining with acid fuchsin and methyl green. The results are shown in figures 32, 33, 34, 35, and 36, in which the mitochondria appear as small red granules. Nearly all these granules are round. A few are slightly elongated, but they are never filimentous.

The pigment, normally yellow in color, may remain unstained or stained only on the surface, as shown in the four large composite granules of figure 32, p. When stained they may take the acid fuchsin or this stain may be replaced by methyl green, depending upon the amount of dichromatization and the length of time the respective stains are allowed to act. In well fixed material it is possible to gauge these factors so that all fuchsin will be driven from the pigment by the methyl green and leave only the mitochondria red as shown in figure 32, c. Intermediate colorizations are, however, frequently obtained in which the pigment has been stained only on the surface of the granule so that the yellow becomes modified either towards the red or the green. These different effects may be obtained on the pigment within a single cell as is shown in figure 32, p and b. But where some of the pigment has taken the red stain, it is usually possible readily to distinguish it from mitochondria, which are much smaller than even the smallest pigment granule. Then too there are a few very small interstitial cells, frequently irregular or slightly elongated in form, which contain no pigment to complicate the picture. In these the mitochondria are exactly the same as those found in the cells that do contain pigment. The mitochondria of the interstitial cells show best in preparations that also exhibit most clearly the mitochondrial content of the neighboring spermatogenic tissue. So it is believed that there is no question about the presence of mitochondria in the interstitial cells both of the ordinary and of the heavily pigmented type.

The suggestions given by Cowdry ('16b) on the causes of failure and the remedy were of great value in discovering the proper time intervals for each block of tissue. An evident source of trouble in earlier attempts to utilize acid fuchsin and methyl green was the excess of fixer left in the tissue by the very rapid washing and dehydration employed. It was necessary to leave some of the acetic-osmic-chromic fixed material for ten minutes in the potassium permanganate. The necessity of apparently excessive fixation was not fully realized, as is apt to be the case with the uninitiated.

As the ordinary interstitial cells enlarge in spring, the mitochondria increase in number simultaneously with the appearance of the large lipoid granules, as shown in figure 33, a, where the lipoid which blackens with osmic acid appears as vacuoles. Many of the mitochondria also appear to become larger. They are most numerous in the perinuclear cytoplasm, but appear through the cytoplasm between the fatty globules and dispersed pigment, the latter being shown in blue in figure 33. As the cells continue to enlarge there appear in the central cytoplasm granules, which are distinctly larger than the mitochondria and many intermediate in size, all of which stain like mitochondria and hence make it difficult to say which are mitochondria and which are not (fig. 34, a). It appears to be a less perfect preservation and staining of these large granules which were described in the first report, where granules of a lipoidal character were shown to be associated with the dense central cytoplasm.

From the presence of intermediate granules and in view of the many accounts of the transformation of mitochondria into secretion granules and various metaplasmic products (cf. Cowdry '16 a), it could easily be believed that the larger fuchsinophil granules have developed from mitochondria; but such a transformation has not been proved in this case. These large fushsinophil granules are more easily preserved and show in preparations not sufficiently well fixed to demonstrate mitochondria. In well fixed tissue, however, all attempts failed to distinguish by color reaction the two types of granules, although the technique permits of considerable selective staining by varying the amount of dechromatization and stain differentiation. In the pancreatic cells, for example, the long filamentous mitochondria situated in the basal part of the cells may be left red while the round zymogen granules nearer the lumen are bluish green.

As shown in figure 34 a, a few of the large fuchsinophil granules may be found at the very periphery of the cell, but nearly all are confined to the central cytoplasm.

In midsummer, usually in July when the enlarged cells are undergoing atrophy (fig. 35), there is an absorption of all of the large fatty globules which occupy mostly the peripheral cytoplasm. In most of the cells many of the large fuchsinophil granules also disappear (fig. 35, a). When atrophy is complete, as is shown in figure 36, none of the typical large fuchsinophil granules are left, though a considerable number of mitochondria persist.

The mitochondria, which are scattered throughout the cytoplasm of the pigment cells, decrease with the gradual disappearance of these cells; but as long as pigment cells are recognizable, mitochondria are demonstrable within them (p of figures 36, 32, 33 and 34).

THE SOURCE OF THE PIGMENT GRANULES

During the midsummer involution of the interstitial tissue, when most of the fuchsinophile granules disappear from many of the cells, there occurs a marked increase in pigmentation of the testis. This is evidently due to a transformation of the fuchsinophil granules that are not absorbed, into a pigment, which is easily differentiated from mitochondria. In figure 35, a, all of these large fuchsinophile granules except three have been so changed that they can be made to take the methyl green while the mitochondria remain red. The three large red granules have not yet become sufficiently changed to be thus distinguished. When the final stage of atrophy is reached some pigment remains in all but a very few of the smallest cells (fig. 36, a, b and c.) This pigment largely persists till hypertrophy commences again in the following spring when it decreases markedly, becomes dispersed in the abundant newly acquired cytoplasm (fig. 33, a), and in most cases disappears entirely.

In the formation of the large pigment cells it appears that the fatty globules which blacken with osmic acid (shown as vacuoles in fig. 34, a) are absorbed as usual and are not transformed bodily into lipochrome as was rather suggested in the earlier report. Simultaneously with this absorption there is a marked increase in the large fuchsinophile granules. In figure 35, b, where is illustrated a transformation stage, the large amount of these fuchsinophil granules is shown just before the last trace of lipoid is gone. To the right of the nucleus are three bluish-green granules. These are pigment. All the fuchsinophil granules finally appear to undergo pigmentation and the result is a cell like p in figure 36, where the pigmentation is shown either in yellow or bluish-green. This particular cell is somewhat larger than usual, showing the size they may attain.

It further appears that the large pigment granules are aggregations of the smaller ones. This is seen in figure 32, p, but is especially evident just after these cells are formed (fig. 36, p). This coalescence becomes more intimate with time so that the individual droplets involved are not seen as is indicated in figures 33, p and 34, p.

COMPARISON OF THE INTERSTITIAL CELLS OF THE TESTIS AND THE OVARY

The foregoing account in connection with the former report on seasonal changes in the testis shows that the interstitial cell cycle, not only in time but also in many cytological details, is somewhat similar in the male and the female woodchuck.

This is easily seen by comparing the right hand row of figures in plate 4 with the parallel arranged left hand row of figures in the same plate. In both cases there is a rapid hypertrophy in the spring after hibernation; but in the ovary this has been preceded by a gradual increase which has been going on during hibernation and sometimes longer; while in the testis there is practically no change till after dormancy. Involution of this tissue is also more gradual in the ovary, where in the case of pregnancy it is going on during April, while in the testis the interstitial cells continue maximal till June or July. The minimal stage is, however reached at about the same time, that is by August. In both cases there is no sudden change with the onset of hibernation, an observation also made by Mann ('16) on the spermophile.

It is, of course, impossible to say with certainty exactly how long the interstitial cells remain hypertrophied in a given individual because tissue cannot be removed from the same animal at various intervals through the year and still leave the animal normal and because the animals involved were captured promiscuously in the field and hence have not a known history. The above comparisons are deduced from the conditions generally prevailing among animals sacrificed at certain periods of the year and showing collateral evidences of being at a given stage in the sexual cycle.

One would be tempted to suggest that the longer hypertrophic stage of the interstitial cells of the testis is related to a longer period of sexual activity in the male. This, however, is not warranted by the presence of ripe spermatozoa for a sufficiently long time nor by the general habits of this species, since they are said to live together in pairs.

During hypertrophy of the interstitial cells of both ovary and testis, the number of mitochondria increase and there appear larger fuchsinophil granules and still larger fatty globules in the cytoplasm. There is considerable individual variation in the relative number of fuchsinophil granules and the larger lipoid bodies—the two constitutents being inversely proportional to each other in both the ovary and the testis.

During atrophy of the interstitial cells of the ovary, the large fuchsinophil granules disappear first leaving large cells that contain only mitochondria and the large fatty globules which are next absorbed. In the testis the fatty matter and large fuchsinophil granules either disappear more or less together or the former is lost first.

In nearly all the interstitial cells of the testis some of the large fuchsinophil granules, and in a smaller number of them apparently all of these granules, become pigment which remains for months to nearly a year. Such pigmentation rarely occurs in the interstitial cells of the ovary.

While most of the interstitial cells of the adult ovary are rarely or only for a short time entirely free from lipoids that blacken with osmic acid, those of the adult testis practically contain such fatty matter only after spring awakening and until July or August.

In the mole a somewhat similar correspondence exists between the duration of hypertrophy of the interstitial cells of the testis and of the ovary, but in both cases the time of hypertrophy does not coincide with the breeding period of early spring and the maximum enlargement of the interstitial cells of the ovary comes somewhat later in the year than in the case of the interstitial cells of the testis. Sufficiently complete descriptions are not given of other animals to make any further comparisons.

As to whether the interstitial cell hypertrophy in either the testis or the ovary is a cause or an effect is debatable. While the presence of the fatty globules and other granules has been taken as evidence of secretory activity, such a conclusion does not necessarily follow. One would expect that if such were the case, the changes in such content would more closely correspond to some particular phase of the sexual cycle than they do, especially in the testis. The interstitial cell activity of the ovary is fairly closely related to ovulation and beginning pregnancy, except in the mole. It would seem, however, very desirable to have the mole as well as many additional species further investigated before drawing conclusions.

An important alternative to the secretory theory of the interstitial cells, is the view that the periodic increase is merely an expression of hyperemia and the general changed metabolism accompanying ovulation and pregnancy. It seems difficult to prove that such is not the case. And, as emphasized by Kingsbury ('14), it is equally hard to exclude the probability that the interstitial cell hypertrophy is related to degenerative changes in the gonads. This is especially true of the theca cells of atretic follicles. Why the interstitial cells not related to follicles are involved is not so well harmonized. Many pathological findings, especially in hermaphrodites, are further difficulties in the way of a satisfactory explanation. In short, the secretory function of the interstitial cells of both the testis and the ovary, while in general assumed, is full of uncertainties. A good statement of the present status of the internal secretary activity of the testis has recently been made by Wheelon ('17).

Neither spermatogenesis nor follicular development are interrupted or modified during atrophy of the interstitial cells, indicating that at least some phases of the primary function of the gonads are independent of interstitial cell activity.

SUMMARY

1. The literature on cyclic changes in the interstitial cells of the ovary is reviewed and indicates in general a hypertrophy of these elements during rut and pregnancy.

2. A marked annual periodicity in the interstitial cells of the ovary of the woodchuck (Marmota monax) is described as consisting of a gradual enlargement during winter followed by a more rapid hypertrophy after hibernation. The cells become three to four times their original diameter. Maximal increase is seen in females that have not become pregnant until late in the breeding season.

3. The increase in the size of the cells is due to an accumulation of lipoid and secretion-like granules in the cytoplasm, though the nucleus also increases. Mitochondria similarly increases in number and some, possibly, in size.

4. Retrogression sets in with the onset of pregnancy and the growth of corpora lutea and continues until July.

5. In the decline the lipoid and large fuchsinophil granules and finally the entire cell disappear in many cases. The cells that survive atrophy retain a number of mitochondria, but the other granules are absorbed.

6. Pigmentary degeneration is rarely observed.

7. The interstitial cells of the ovary are minimal in size during late summer and early autumn, but tend soon to acquire lipoid and slowly to enlarge.

8. During late involution of the interstitial cells the follicles show a tendency to grow. By autumn a number of fairly large Graafian follicles and many smaller ones are evident.

9. The origin of the interstitial cells of the ovary is briefly discussed and shown to be regarded by the vast majority as having come from connective tissue, or stroma of the ovary, either directly or indirectly through the theca interna of atretic follicles. A few investigators insist on an epithelial origin.

10. Proliferation of the germinal epithelium in adult ovaries indicates strongly a formation of new interstitial cells from elements that migrate into the ovary from its covering epithelium. This is most noticeable from late in pregnancy till late summer.

11. Transformation of stroma cells into interstitial cells in adult animals is also evident. There appears to be no difference between the hypertrophied cells of the theca interna of atretic follicles and interstitial cells that are not related to follicles.

12. The persistence of full-sized corpora lutea of pregnancy until many weeks after parturition and the appearance of an abundance of fatty globules in the lutein cells before they decrease in size was observed. All corpora lutea have disappeared by September.

13. A restudy of the interstitial cells of the testis of the woodchuck is included. The presence of mitochondria in the form of round granules in all types of interstitial cells is reported. These mitochondria increase in number and some also in size during hypertrophy.

14. A better demonstration of the small lipoid granules of the central cytoplasm shows that they have many of the staining reactions of mitochondria but are distinctly larger in size.

15. These small granules and not the larger and more peripherally placed fatty globules are believed to be the source

of the lipochrome, or pigment, which is formed during atrophy of the interstitial cells of the testis.

16. The large pigment granules are formed by the coalescence of smaller ones.

17. The general similarity in the interstitial cells of the testis and of the ovary is shown. The main differences between them are the more sudden hypertrophy and subsequent atrophy that occur in those of the testis and the almost entire absence of pigmentary degeneration in those of the ovary.

My thanks are due to Dr. B. F. Kingsbury of Cornell University for suggesting the problem and for counsel during the prosecution of the work; and to Dr. Sutherland Simpson of the department of Physiology for generous aid in procuring material. I take this opportunity also of thanking Dr. C. M. Jackson for the privilege of carrying out the major part of the histological work at the Institute of Anatomy in the University of Minnesota.

LITERATURE CITED

Aimé, P. 1907 Recherches sur les cellules interstitielles de l'ovaire chez quelques mammifères. Thèse. Nancy. Arch. de Zool. expér. et génér., Série 4, T. 7, p. 95.

Allen, B. M. 1904 The embryonic development of the ovary and testis of the mammals. Am. Jour. Anat. vol. 3, p. 89.

Anzilotti 1909 Contributo sperimentale allo studio della cosidetta glandula interstiziale dell'ovaio. Ann. di Ostet. e Ginec. (Cited by Pardi '14a.)

Aschner, B. 1914 Über Morphologie und Funktion des Ovariums unter normalen und pathologischen Verhältnissen. Arch. f. Gynaek., Bd. 102, p. 446.

Athias, M. 1911 Observations cytologiques sur l'ovaire des mammifères. I. Les cellules interstitielles de l'ovaire chez le cobaye (foetus à terme et nouveau-né). Anat. Anz., Bd. 39, p. 238.

—— 1912 L'appareil mitochondrial des cellules interstitielles de l'ovaire du murin. Compt. Rend. Soc. de Biol., T. 73, p. 448.

—— 1916 Sur le déterminisme de l'hyperplasie de la glande mammaire et de la sécrétion lactée. Compt. Rend. Soc. de Biol., T. 79, p. 557.

Balfour, F. M. 1878 On the structure and development of the vertebrate ovary. Quart. Jour. Micr. Sc., N. S., vol. 18, p. 383.

Basso, G. L. 1906 Modificazioni istologiche delle ovaie in gravidanza con speciale riguardo alla theca interna dei follicoli atresici. Raccolta di scritti pel Giubileo didottico del prof. Mangiagalli.

Bayer, H. 1908 Anatomie der weiblichen Geschlechtsorgane. Vorlesungen über Allgemeine Geburtshülfe. Bd. 1, Heft 3, p. 259. (cf. p. 500.)

Bell, W. Blair 1916 The Sex Complex. London, 1916.

Benthin, W. 1911 Über Follikelatresie in Säugetierovarien. Arch. f. Gynaek., Bd. 94, p. 599.

Bianchi, D. C. 1907a Osservázioni sulla struttura a sulla funzione della cosidetta ''ghiandola interstiziale del' ovaia.'' Arch. di Fisiol., vol. 4, p. 523.

—— 1907b Osservazioni sull'modo di compostarsa della ghiandola interstitiale dell'ovaio negli animali ibernanti. Boll. de Soc. med.-chir. di Pavia, p. 222.

Biedl, A. 1913 Innere Sekretion, 2 Auflage, II. Teil, pp. 313-339.

Boring, A. M., and Pearl, R. 1917 Sex studies. IX. Interstitial cells in the reproductive organs of the chicken. Anat. Rec., vol. 13, p. 253.

Böshagen, A. 1904 Über die verschiedenen Formen der Rückbildungsprodukte der Eierstocksfollikel und ihre Beziehungen zu den Gefässveränderungen des Ovariums. Zeitschr. d. Geburtsh. u. Gynaek., Bd. 53, p. 323.

Bouin, M. 1900 Histogenèse de la glande génitale femelle chez Rana temporaria. Arch. d. Biol., T. 17, p. 201. (Thèse de la Fac. des Sc. de Nancy, 1900.)

Bouin, P., et Ancel, P. 1903 Recherches sur les cellules interstitielles du testicule des mammifères. Arch. d. Zool. expér., Ser. 4, T. 1, p. 437.

—— 1909 Sur les homologies et la signification des glandes à sécretion interne de l'ovaire. Compt. Rend. Soc. de Biol., T. 67, p. 464, 497.

Branca, A. 1903. Le testicule chez certains animaux en captivité. Compt. Rend. Assoc. Anat., session 5, p. 193.

v. d. Broek, A. J. P. 1910 Entwicklung und Bau des Urogenital-Apparates der Beutler und dessen Verhältniss zu diesen Organen andrer Säuger und niedern Wirbeltiere. Morph. Jahrb., Bd. 41, p. 437.

Cattaneo (cited by Pardi '14b).

Child, C. M. 1897 Centrosome and sphere in cells of the ovarian stroma of mammals. Zool. Bull. (now the Biol. Bull.), vol. 1, p. 87. (A shorter account is found in Science, N. S., 1897, vol. 5, p. 231.)

Ciaccio, C. 1910 Contributo alla distribuzione ed alla fisiopatologia cellulare dei lipoidi. Arch. f. Zellforsch., Bd. 5, p. 235.

Ciulla 1909 Lo ghiandole á secrezione interna in gravidanza. Palermo. (Cited by Pardi '14b.)

Clark, J. G. 1898 Ursprung, Wachstum und Ende des Corpus luteum nach Beobachtungen am Ovarium des Schweines und des Menschen. Arch. f. Anat. u. Physiol., Anat. Abt., p. 95.

Coert, H. J. 1898 Over de Ontwikkeling en den Bouw van de Geschlachtsklier bij de Zoogdieren meer in het bejzonder van den Eierstok. Inaug. Diss., Leiden.

Cohn, F. 1903 Zur Histologie und Histogenese des Corpus luteum und des interstitiales Ovarialgewebes. Arch. f. mikr. Anat., Bd. 62, p. 745.

—— 1909 Über das Corpus luteum und den atretischen Follikel des Menschen und deren cystische Derivate. Arch. f. Gynaek., Bd. 87, p. 367.

Corner, G. W. 1915 Corpus luteum of pregnancy as it is in swine. Contributions to Embryology, vol. 2 (Publication No. 222, Carnegie Institution, Washington), No. 5, p. 71.

Cowdry, E. V. 1916a The general functional significance of mitochondria. Am. Jour. Anat., vol. 19, p. 423.

—— 1916b The structure of chromophile cells of the nervous system. Contributions to Embryology, vol. 4 (Publication No. 224, Carnegie Institution, Washington), No. 11, p. 29.

Cushing, H., and Goetsch, E. 1915 Hibernation and the pituitary body. Jour. Exper. Med., vol. 22, p. 25.

Drips, Della G. 1917 Studies on the ovary of the spermophile; with special reference to the corpus luteum. Thesis, University of Minnesota. Jour. of Morph. (in press).

—— 1918 Studies on the ovary of the spermophile with special reference to the corpus luteum. Anat. Rec., vol. 14, p. 34.

Dubreuil, G. 1911 Les mitochondries des cellules adipeuses. Compt. Rend. Soc. de Biol., T. 70, p. 48; Transformation directe des mitochondries et des chondriocontes en graisse dans les cellules adipeuses. Ibid., p. 264.

Duesberg, J. 1918. On the interstitial cells of the testicle in Didelphys. Biol. Bull., vol. 35, p. 175.

Falta, W. 1916 The Ductless Glandular Diseases. Translated and Edited by M. K. Meyer, 2nd Ed., p. 370.

Fellner, O. 1909 Zur Histologie des Ovariums in der Schwangerschaft. Arch. f. mikr. Anat., Bd. 73, p. 288.

Firket, J. 1914 Recherches sur l'organogenèse des glandes sexuelles chez les oiseaux. Arch. d. Biol., T. 29, p. 201.

Foster, L. 1917 The Ovaries and Mental Disease. Proc. Roy. Soc. Med., vol. 10, Section of Psychiatry, p. 65.

Fraenkel, L. 1905 Vergleichende histologische Untersuchungen über Vorkommen drüsiger Formationen im interstitiellen Eierstocksgewebe (glande interstitielle de l'ovaire). Arch. f. Gynaek., Bd. 75, p. 443.

—— 1911 Die interstitielle Eierstocksdrüse. Berl. klin. Wochenschr. (48 Jahrg., 1 Halbj.), p. 60.

Ganfini, C. 1907 Sul probabile significato fisiologico dell-'atresia follicolare nell'ovaio di alcuni mammiferi. Arch. Ital. di Anat. et Embriol., vol. 6, p. 346.

—— 1908 Sulla struttura e sviluppo delle cellule interstiziali dell'ovajo. Arch. Ital. di Anat. et Embriol., vol. 7, p. 373.

Gley, E. 1917 The Internal Secretions: Their Physiology and Application to Pathology. .Trans. by Maurice Fishberg. Paul B. Hoeber, New York.

Greggio, E. 1910 Intorno alle modificazioni strutturali dell' ovaio in alcuni processi morbosi ed in alcune particolari condizioni fisiologiche. Arch. Ital. di Ginec., vol. 13, p. 1.

Harz, W. 1883 Beiträge zur Histologie des Ovariums der Säugethiere. Arch. f. mikr. Anat., Bd. 22, p. 374.

His, W. 1865 Beobachtungen über den Bau des Säugetiereierstocks. Arch. f. mikr. Anat., Bd. 1, p. 151.

Janosik, J. 1885 Histologisch-embryologische Untersuchungen über des Urogenitalsystem. Sitzungsber. d. Kais. Akad. d. Wiss., Wien, Math.-Naturw. Kl., Abt. 3, p. 97.

—— 1888 Zur Histologie des Ovariums. Sitzungsber. der Kais. Akad. d. Wiss., Wien, Bd. 96, p. 172.

Jordan, H. E. 1911. The spermatogenesis of the opossum (Didelphys virginiana) with special reference to the accessory chromosome and the chondriosomes. Arch. f. Zellforsch., Bd. 7, p. 41.

Keller, R. 1913 Ueber Veränderungen am Follikelapparat des Ovariums während der Schwangerschaft. Beitr. z. Geburtsh. u. Gynaek. Leipzig. Bd. 19, p. 13.

Kingery, H. M. 1917 Oogenesis in the white mouse. Jour. of Morph., vol. 30, p. 261.

Kingsbury, B. F. 1914 The interstitial cells of the mammalian ovary: Felis domestica. Am. Jour. Anat., vol. 16, p. 59.

von Kölliker, A. 1898 Über Corpora lutea atretica bei Säugetieren. Verh. d. Anat. Gesell. zu Jena. Ergänzungsh. zu Bd. 14, Anat. Anz., p. 149.

Lane-Clapon, J. E. 1906 On the origin and life history of the interstitial cells of the ovary in the rabbit. Proc. Roy. Soc. London, Series B, vol. 77, p. 32.

Levi, G. 1913 Note citologiche sulle cellule somatiche dell' ovaio dei mammiferi. Arch. f. Zellforsch., Bd. 11, p. 516; Arch. Ital. de Biol., 1914, T. 61, p. 129.

Leydig, F. 1857 Lehrbuch der Histologie des Menschen und der Thiere. Hamm, G. Grote'sche Buchhandlung, p. 507.

Limon, M. A. 1902 Étude histologique et histogénique de la glande interstitielle de l'ovaire. Arch. d'Anat. micr., T. 5, p. 155.

Loeb, L. 1911 Untersuchungen über die Ovulation nebst einigen Bemerkungen über de Bedeutung der sogenannten ''interstitiellen Drüse'' des Ovariums. Zentralbl. f. Physiol., Bd. 25, p. 336.

—— 1914 The correlation between the cyclic changes in the uterus and ovaries in the guinea pig. Biol. Bull., vol. 27, p. 1.

—— 1917 The relation of the ovary to the uterus and mammary gland from the experimental aspect. Surg., Gynec. and Obst., vol. 25, p. 300.

—— 1918 The relations between the interstitial gland of the testicle, seminiferous tubules and the secondary sexual characters. Biol. Bull., vol. 34, p. 33.

M'Ilroy, Louise 1917 Physiology of the female reproductive organs. New System of Gynecology, edited by Eden and Lockyer, London, vol. 1, p. 39.

MacLeod, M. J. 1880 Contribution a l'étude de la structure de l'ovaire des mammifères. Arch. d. Biol., T. 1, p. 241.

Mann, F. C. 1916 The ductless glands and hibernation. Am. Jour. Physiol., vol. 41, p. 173.

Meyer, R. 1913 Ueber die Beziehung der Eizelle und des befruchteten Eies zum Follikel-apparat, sowie des Corpus luteum zur Menstruation. Arch. f. Gynaek., Bd. 100, p. 1.

Montuoro, F. 1903 Sulle cellule midollari dell'ovaio del coniglio. Con tavola IV. Arch. Ital. d. Anat. ed. Embr., vol. 2, p. 45.

Mulon, P. 1910 Sur une sécrétion lipoide nouvelle de la glande interstitielle ovarienne. Compt. Rend. Soc. Biol., T. 69. p. 423.

Nussbaum, M. 1880 Zur Differenzierung des Geschlechts in Thierreich. Arch. f. mikr. Anat., Bd. 18, p. 1.

O'Donoghue, C. H. 1916 On the corpora lutea and interstitial tissue of the ovary in the Marsupialia. Quart. Jour. Micr. Sci., vol. 61, p. 433.

Paladino, G. 1887 Ulteriori ricerche sulla distruzione e rinnovamento continuo del parenchima ovarico nei mammiferi. Napoli. (230 pp., 9 pl.).

—— 1888 La destruction et le renouvellement continuel du parenchyme ovarique des mammifères. Arch. Ital. de Biol., T. 9, p. 176.

Pardi, U. 1914a Sur la fonction endocrine de l'ovaire durant la gestation. Lo Sperimentale, p. 183; Arch. Ital. d. Biol., T. 61, p. 177.

—— 1914b Sur les cellules interstitielles ovariques de la lapine et sur les éléments de la thèque interne de l'ovaire humain hors de la gestation et durant celle-ci. Lo Sperimentale, p. 409; Arch. Ital. d. Biol., T. 62, p. 353.

Pearl, R., and Boring, A. M., 1918 Sex studies. X. The corpus luteum in the ovary of the domestic fowl. Am. Jour. Anat., vol. 23, p. 1.

Pinto, C. 1905 Note istologiche sulle modificazioni delle ovaia in gravidanza. Ann. di Ostet. e Ginec., vol. 27, p. 476; Boll. d. Soc. med.-chir. di Pavia, p. 146.

Plato, J. 1897 Zur Kenntniss der Anatomie und Physiologie der Geschlechtsorgane. Arch. f. mikr. Anat., Bd. 50, p. 640.

Popoff, N. 1911 Le tissue interstitiel et les corps jaunes de l'ovaire. Arch. de Biol., T. 26, p. 483.

Pottet, M. 1910 Contribution à l'étude du corps jaune pendant la grossesse. Thèse, Paris.

Rabl, H. 1898 Beitrag zur Histologie des Eierstocks des Menschen und der Säugetiere nebst Bemerkungen über die Bildung von Hyalin und Pigment. Anat. Hefte, Bd. 11, p. 109.

Rasmussen, A. T. 1915 The oxygen and carbon dioxide content of the blood during hibernation in the woodchuck (Marmota monax). Am. Jour. Physiol., vol. 39, p. 20.

—— 1917 Seasonal changes in the interstitial cells of the testis in the woodchuck (Marmota monax). Am. Jour. Anat., vol. 22, p. 475.

Regaud, C. et Dubreuil G. 1906 Richerches sur les cellules interstitielles de l'ovaire chez le lapin. Bibliogr. Anat., T. 15, p. 169.

—— 1907 Variations macroscopiques de la glande interstitielle de l'ovaire chez la lapine. Compt. Rend. Soc. Biol., Paris, T. 63, p. 780.

—— 1908 Parallélisme des variations macroscopiques et microscopiques de la glande interstitielle dans l'ovaire de la lapine. Compt. Rend. Soc. Biol., T. 64, p. 901.

—— 1909 Nouvelles recherches sur les modifications de la glande interstitielle de l'ovaire consécutives á l'isolement et à la cohabitation avec le mâle. Compt. Rend. Soc. Biol., Paris, T. 67, p. 348. (An analysis of the work of Regaud and Dubreuil has been made by Pottet in Ann. d. Gynécol. et D'Obstét., 1910, Série 2, T. 7, p. 363.)

Regaud, C. et Policard A. 1901 Notes histologiques sur l'ovaire des mammifèeres. Compt. Rend. Assoc. des Anat., Session 3, Lyon, p. 45.

Saimont, G. 1905 Recherches relatives à l'organogenèse du testicule et de l'ovaire chez le chat. Arch. de Biol., T. 22, p. 71.

Schaffer, A. 1911 Vergleichende histologische Untersuchungen über die interstitielle Eierstochsdrüse. Arch. f. Gynaek., Bd. 74, p. 491.

Schottländer, J. 1891 Beiträge zur Kenntniss der Follikelatresie nebst einigen Bemerkungen über die unveränderten Follikel in den Eierstocken der Säugetiere. Arch. f. mikr. Anat., Bd. 37, p. 192.

—— 1893 Über den Graafschen Follikel, seine Entstehung beim Menschen und seine Schicksale beim Menschen und Säugetieren. Arch. f. mikr. Anat., Bd. 41, p. 219.

—— 1914 Zur Theorie der Abderhalden'schen Schwangerschaftsreaktion, sowie Anmerkungen über die innere Sekretion des weiblichen Genitales. Erwägungen auf morphologischer Grundlage. Zentralbl. f. Gynaek., Jahrgang 38, p. 425.

Schulin, K. 1881 Zur Morphologie des Ovariums. Arch. f. mikr. Anat., Bd. 19, p. 442.

Scott, W. J. M. 1916 Experimental mitochondrial changes in the pancreas in phosphorus poisoning. Am. Jour. Anat., vol. 20, p. 237.

Seitz, L. 1906 Die Follikelatresie während der Schwangerschaft, insbesondere die Hypertrophie und Hyperplasie der Theca-interna-Zellen (Theca-lutein-Zellen) und ihre Beziehungen zur Corpus luteumbildung. Arch. f. Gynaek., Bd. 77, p. 203.

De Sinety, L. 1877 De l'ovaire pendant la grossesse. Compt. Rend. de l'Acad. d. Sci., Paris, T. 85, p. 345.

Tandler, J. und Grosz, S. 1911 Über den Saisondimorphismus des Maulwufhodens. Arch. f. Entw. der Organ, Bd. 33, p. 297.

—— 1913 Die biologischen Grundlagen der sekundären Geschlechtscharaktere. Berlin.

Tourneux, G. 1879 Des cellules interstitielles de l'ovaire. Journ. de l'Anat. et de la Physiol., T. 15, p. 305.

Van der Stricht, O. 1901 L'atrésie ovulaire et l'atrésie folliculaire du follicule de Graaf, dans l'ovaire du chauve-souris. Verh. d. Anat. Gesell. zu Bonn, p. 108.

—— 1912 Sur le processus de l'excrétion des glandes endocrines: Le corps jaune et la glande interstitielle de l'ovaire. Arch. de Biol., T. 27, p. 585.

Wagener, G. R. 1879 Bemerkungen über den Eierstock und den gelben Körper. Arch. f. Anat. u. Entw. (Reichert's Archiv), p. 175.

Wallart, J. 1907 Untersuchungen über die interstitielle Eierstocksdrüse beim Menschen. Arch. f. Gynaek., Bd. 81, p. 271.

—— 1908 Untersuchungen über das Corpus luteum und die interstitielle Eierstockdrüse während der Schwangerschaft. Zeitschr. f. Geburtsh. u. Gynaek., Bd. 63, p. 520.

Wheelon, H. 1917 The internal secretion of the testis. Interstate Med. Jour. (St. Louis), vol. 24, p. 1089.

Whitehead, R. H. 1912 On the chemical nature of certain granules in the interstitial cells of the testis. Am. Jour. Anat., vol. 14, p. 63.

Von Winiwarter, H. 1908 Das interstitielle Gewebe der menschlichen Ovarien. Anat. Anz., Bd. 33, p. 1.

—— 1912 Observations cytologiques sur les cellules interstitielles du testicule humain. Anat. Anz., Bd. 41, p. 309.

Von Winiwarter, H. et Sainmont, G. 1908 Über die ausschliesslich postfetale Bildung der definitiven Eier bei der Katze. Anat. Anz., Bd. 32, p. 613.

Wolz, Elisabeth 1912 Untersuchungen zur Morphologie der interstitiellen Eierstocksdrüse des Menschen. Arch. f. Gynaek., Bd. 97, p. 131.

Zalla, M. 1907 Ricerche sopra la struttura e l'istogenesi della sostasnza midollare dell'ovaia. Arch. Ital. d. Anat. e d. Embr., vol. 6, p. 706.

Plate 1

EXPLANATION OF FIGURES

1. Photograph of longitudinal section of ovary of active adult woodchuck; just before hibernation (Oct. 26). Meves' acetic-osmic-chromic fixer, 10 micra, no stain, no cover. X 7. Shows distribution of interstitial cells, which are co-extensive with the blackened lipoid, which is unusually abundant for this time of the year.

2. Photograph of adult ovary, comparable to figure 1 in thickness, plane of section and technique. X 7. Animal taken from normal habitat while dormant. Typical ovary of late hibernation (Feb. 25). Shows an increase in interstitial tissue as judged from the amount of lipoid.

3. Photograph, comparable to figures 1 and 2, showing extreme hypertrophy of interstitial cells as met with late in the rutting season (last of April and first of May). X 7. Somewhat greater development than normally occurs at the beginning of pregnancy earlier in the season. CL are young corpora lutea. CL′ are corpora lutea of earlier ovulation not followed by pregnancy.

4. Photograph of area outlined in white in figure 3 under higher magnification. X 30. Shows the extent of lipoid-laden interstitial cells in the stroma.

5. Photograph comparable in every way to figures 1, 2 and 3, showing in black the characteristic interstitial cell content of late pregnancy and early lactation (May 8). X 7.

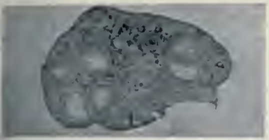
6. Photograph of typical ovary of midsummer (July 28) after interstitial cells have nearly reached a minimum. Shows characteristic fatty content of corpora lutea just before they commence rapidly to disappear. Same technique as in figures 1, 2, 3 and 5. X 7.

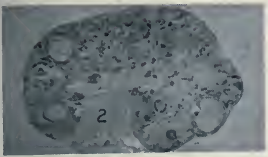
7. Photograph of typical ovary of late summer when interstitial cells are minimal in size and number and almost free from lipoid. The single dark area is the remains of a corpus luteum which has not yet entirely disappeared. Same technique as other general views in this plate. Aug. 30. X 7.

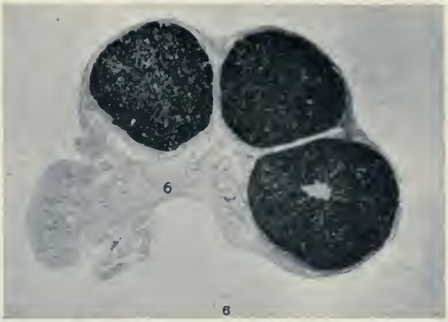
8. Photograph of ovary of adult animal isolated in captivity till May 18, showing the small size of the ovary and the relative amount of interstitial tissue in females kept in captivity during the spring. Comparable to other general views in this plate. X 7.

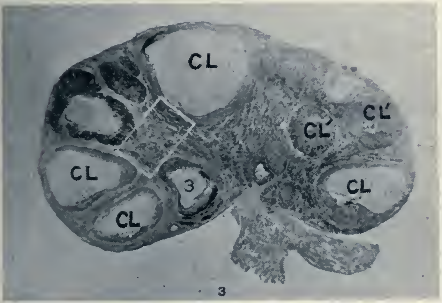
9. Photograph showing migration of interstitial cells (identifiable by their black lipoid content and indicated by the arrow, which also shows the direction of movement) with neighboring stroma into a portion of a young corpus luteum. A portion of the corpus hemorrhagicum (CH) is present. Same technique as in preceding figures. X 60.

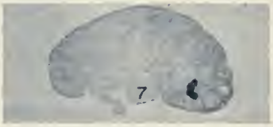
10. Photograph showing relation of interstitial cells (dark areas) to vascular channels, which appear as prominent light areas because the blood has been washed out. In the center of each interstitial cell mass is usually a medullary cord. Zenker's with only 4 drops of acetic acid per 100 c.c., 5 micra, copper hematoxylin. X 45.

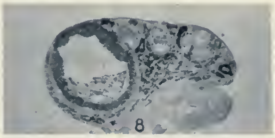


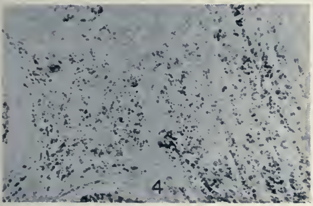

2

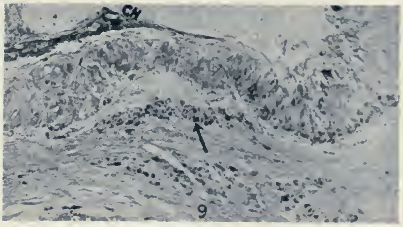

6
6

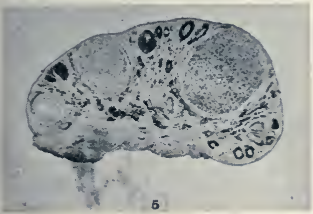

CL
CL'
CL'
CL
3
CL
CL
3


7


8


4


CH
9


5

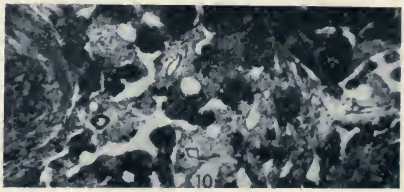

10

Plate 2

EXPLANATION OF FIGURES

11. Photograph of ovary of pregnant woodchuck (foetuses 40 mm. to 45 mm. in length). Shot in the field April 17. Zenker's, 10 micra, iron hematoxylin. X 67. Shows general view of cortex where cells appear to enter the ovary from the germinal epithelium.

12. Photograph of portion of figure 11 under higher magnification. X 333.

13. Photograph of peripheral portion of section of ovary of old adult showing cells leaving germinal epithelium. Early autumn (Sept. 5). The uninterrupted portion of the tunica albuginea is marked T. Carnoy's, 4 micra, iron hematoxylin. X 333.

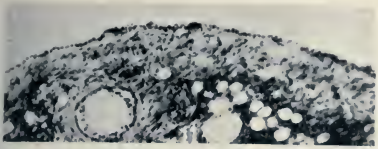
14. Photograph of another portion of same section as figure 13 showing tunica albuginea apparently interrupted by cells from the germinal epithelium passing through it. A mitotic figure is seen at M. X 333.

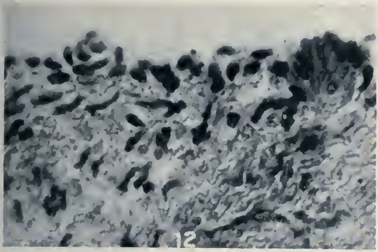
15. Photograph of portion of adult ovary, in which the corpora lutea are rapidly being absorbed and many follicles are developing (Aug. 16), showing close relation of cells with oval and hyperchromatic nucleus to young follicles. Carnoy's, 3 micra, iron hematoxylin. X 333.

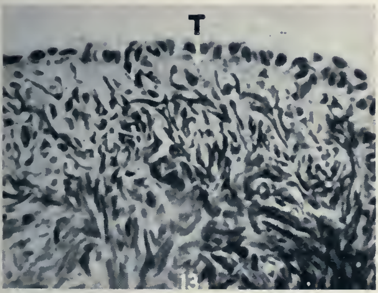
16. Photograph of medullary stroma of adult ovary as it appears in early autumn, showing relatively few cells with round vesicular nuclei. Carnoy's 4 micra, iron hematoxylin. X 200.

17. Photograph of medullary stroma of adult ovary as it appears at the close of hibernation (Mar. 18). Animal sacrificed within 48 hours after waking up. To be compared with figure 16. Shows a noticeable increase in size and number of round nuclei (nuclei of interstitial cells). Carnoy's, 4 micra, iron hematoxylin. X 200.

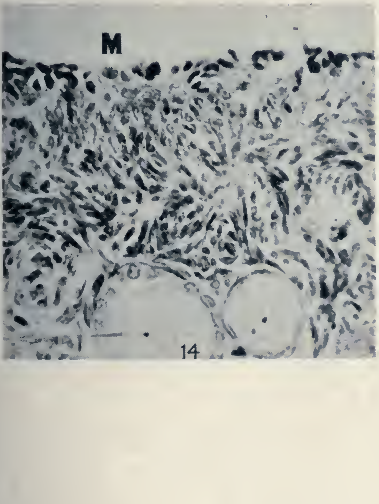
18. Photograph of medullary stroma of adult ovary when interstitial cells are maximal. Note large size of cells and vesticular nuclei. Carnoy's, 4 micra, iron hematoxylin. X 200.


11

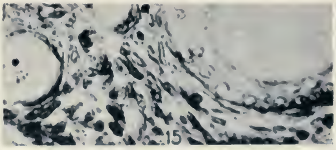

12

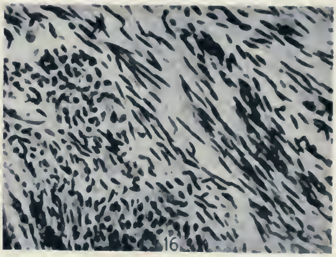


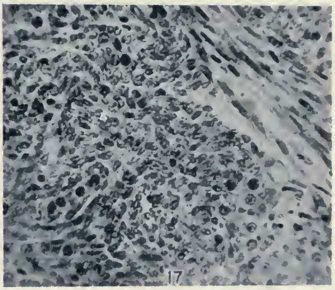
13



14


15


16


17

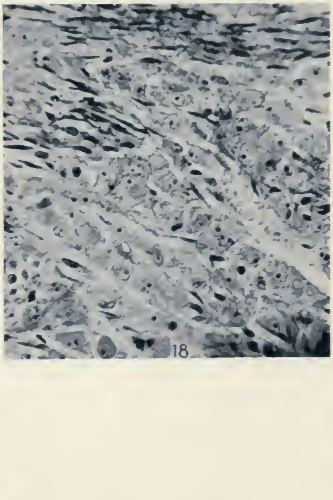

18

Plate 3

EXPLANATION OF FIGURES

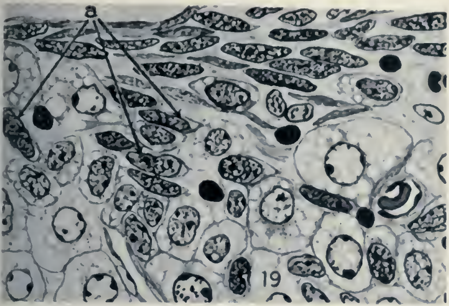
19. Camera lucida drawing of stroma of adult ovary showing transitional stages between elongated cells (free from fatty globules, with hypercromatic nucleus and with but little cytoplasm which is difficult to demonstrate) and typical interstitial cells with lipoid-laden cytoplasm (indicated as vacuoles) and round vesicular nucleus. Zenker's with only 4 drops of acetic acid per 100 cc., 4 micra, iron hematoxylin. X 800. a, early indications of cytoplasmic increase. Solid stained nuclei are lymphocytes.

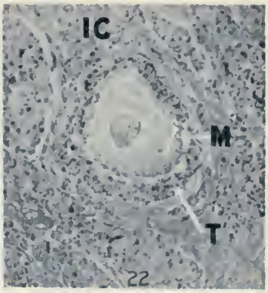
20. Photograph of a corpus luteum-like structure still containing an ovum (O). Early pregnancy (uterine enlargements 8 mm. to 9 mm. in diameter, April 27). Zenker's, 10 micra, iron hematoxylin. X 30.

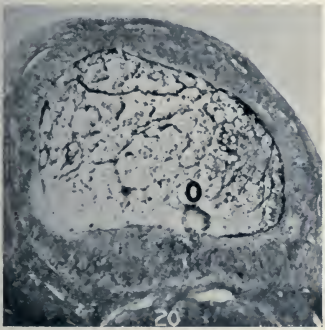
21. Camera lucida drawing of portion of adult ovary during midsummer when corpora lutea (CL) are degenerating, showing characteristic down growths of germinal epithelium, giving rise to the irregular masses and cords of cells shown in the region marked C. Tunica albuginea is dark. X 500.

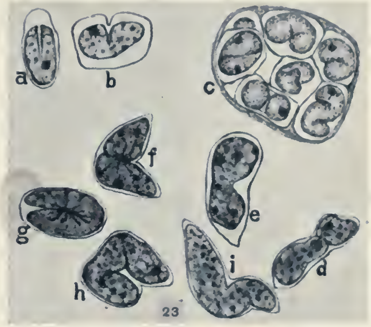
22. Photograph of atretic follicle and adjacent stroma when interstitial cells (IC) are maximal. M indicates the basement membrane of the granulosa and T the theca interna. Lipoid is black. Acetic-osmic-chromic fixer, 5 micra, acid fuchsin, no cover. X 44.

23. Camera lucida drawings, showing certain nuclear characteristics. Carnoy's, 5 micra, iron hematoxylin. a and b are from the germinal epithelium of pregnant female and show what might be regarded as indications of direct cell division, but which is believed to be merely a folding. X 1000. c is a section through cell cord newly derived from the germinal epithelium, showing the tendency for the nuclei to fold and twist. X 1000. d, e, f, g, h and i are cells encountered in the stroma especially in midsummer, showing a variety of forms they may assume. Nucleus is granular and hyperchromatic. Appear to have been derived from the germinal epithelium and may transform into typical interstitial cells. X 1500.


a
19


IC
M
T
22


O
20


a
b
c
f
e
g
i
d
h
23

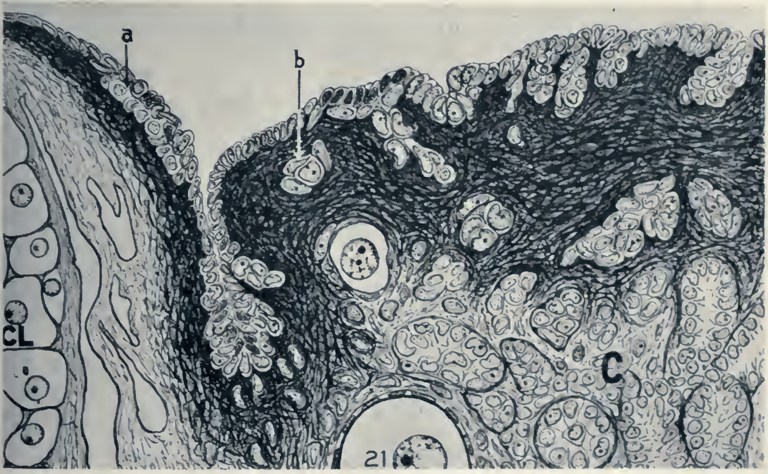

a
b
CL
C
21

Plate 4

EXPLANATION OF FIGURES

All figures in this plate X 1000. Acetic-osmic-chromic or neutral formalin-dichromate fixation, 3 micra, acid fuchsin and methyl green stain. Large fatty globules appear black or as vacuoles. Mitrochondria and larger (secretion?) granules are red. Nucleolus either blue or red.

24. Types of interstitial cells of ovary in autumn before hibernation. a and c are transitional stages between elongated cells in the stroma and typical interstitial cells. Red granules are mitochondria.

25. Largest interstitial cells of ovary found immediately after waking up from hibernation. a is an intermediate stage between cells of figure 24 and of figure 26. Note appearance of a few red granules distinctly larger than mitochondria.

26. Interstitial cells of ovary at maximum hypertrophy during spring. a is a group of four cells with indistinct boundaries. Note numerous large and intermediate fuchsinophil granules.

27. Successive stages in atrophy of interstitial cells of ovary (late spring and early summer).

28. Interstitial cell (of ovary) that has survived atrophy (midsummer).

29. Lutein cell for comparison with interstitial cells. Shows typical granular cytoplasm.

30. Intermediate stages between ordinary stroma cells and interstitial cells of ovary.

31. Stages in degeneration of lutein cells for comparison with interstitial cells.

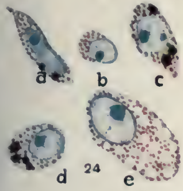
32. Interstitial cells of testis as they are in autumn. b contains three pigment granules, one yellow (natural color), one red and one blue. Pigment in c is blue. p is a "pigment cell," showing nucleus crowded to lower border of cell and cytoplasm filled with pigment granules, which are mostly blue, four red, one orange and four yellow (natural color) stained red only on the surface. Shows coalescence of smaller granules to form larger ones. Mitochondria appear as small red granules.

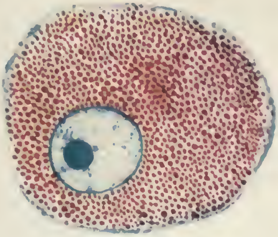
33. a is an interstitial cell of testis in early stage of spring hypertrophy. p is a pigment cell of this stage. Fatty globules appear as vacuoles, pigment blue, mitochondria red.

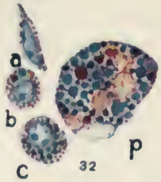
34. a is an interstitial cell of testis during maximum hypertrophy in spring and early summer. Pigment (blue) nearly absent. There are many fuchsinophil granules (red) and large fatty globules (vacuoles). The group arrangement often seen is indicated. p is a pigment cell of this time of the year just before complete atrophy. Mitochondria red. Pigment blue.

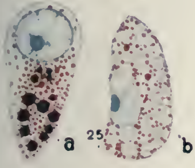
35. Interstitial cells of testis during midsummer atrophy. b is destined to become a pigment cell as p of next figure. Newly formed pigment is blue. Small red granules are mitochondria.

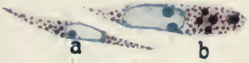
36. Interstitial cells of testis just after atrophy (Aug. 5). a represents the majority and contains some pigment (blue) and mitochondria (red). p is a newly formed pigment cell. Mitochondria are red and pigment is blue or yellow (natural color) stained only on the surface. Formation of larger granules from coalescence of smaller ones is evident.

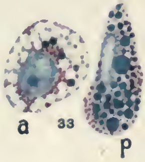

24

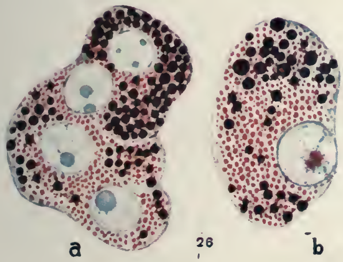

29

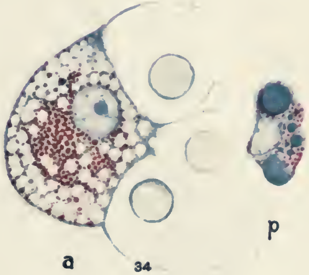

32

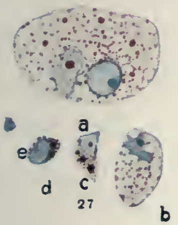

25


30

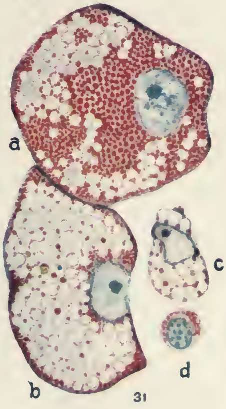

33

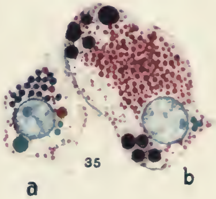

26

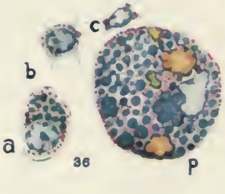

34


27

28


31


35


36

No. 13 37

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, SEPTEMBER 29

Reprinted from THE JOURNAL OF COMPARATIVE NEUROLOGY, Vol. 31, No. 1, October, 1919

THE MITOCHONDRIA IN NERVE CELLS DURING HIBERNATION AND INANITION IN THE WOODCHUCK (MARMOTA MONAX)

A. T. RASMUSSEN

Institute of Anatomy, Medical School, University of Minnesota

CONTENTS

Introduction 37
Material and methods 39
Results and discussion 42
Summary 47

INTRODUCTION

The discovery of mitochondria (chondriosomes) in practically all living cells—except possibly in the simple blue-green algae, Cyanophyceae, and in most bacteria—and the striking similarity between those in plants and those in animals, add much interest to the question of the rôle played by these cytoplasmic granules, as is evident from the increasing number of papers appearing on the various phases of the subject. Since excellent general discussions are found in many publications, none will be attempted in this brief report. Four of the more recent papers, by E. V. Cowdry ('16 a, '18), N. H. Cowdry ('17), and by Duesberg ('19), taken together cover the subject most admirably. E. V. Cowdry's 1918 article is unusually comprehensive. It is fully evident from the literature that the function of mitochondria is a much disputed question. The widespread distribution and great similarity wherever found naturally suggest some close connection with fundamental cell processes.

Alterations in the number, size, shape, and staining reaction as a result of degrees of cell activity has been described to some extent especially in glands. But the literature on the correspondence between mitochondria and functional states in nervous tissue is extremely limited. Strongman ('17), who investigated the results of muscular fatigue in white mice, found no constant variation due to such activity, except possibly a tendency toward a clumping together of the mitochondria in the fatigued animals. This effect was especially apparent at the base of the large dendrite of Purkinje cells in the cerebellum. Luna ('13) found that shortly after cutting a large peripheral nerve trunk in the toad the mitochondria of corresponding ganglion cells lost their regular distribution, increased in size, and showed an increased affinity for the iron-haematoxylin stain. In more advanced stages of degeneration the mitochondria disappeared entirely. Busacca ('15) found that stimulation of the eye of the toad with light caused a decrease in the number of mitochondria in the pigment cells of the retina.

Earlier observations on the behavior of neurosomes—the smaller of which have been shown by E. V. Cowdry ('12) to be mitochondria—made by Levi ('96), Motta-Coco and Lombardo ('03), and by Scott ('05) indicate an increase in the number of these fuchsinophil granules during activity in nerve cells. Scott suggests, however, that such changes may be only apparent, since the entire cell is not examined, but merely the cell body, while the most important point of activity is at the nerve endings.

On the whole, it appears that mitochondria of nerve cells are comparatively resistant to conditions associated with normal processes.

In many pathological tissues (for literature see McCann, '18, and E. V. Cowdry, '18) mitochondria often show marked alterations even in early stages, and yet, according to McCann ('18), in experimental poliomyelitis the mitochondria persist in normal number in proportion to the cytoplasm in cells not only where the Nissl substance has disappeared, but also in the latest stages of neurophagocytosis.

Having seen nothing on record concerning mitochondria in nerve cells during hibernation, it seemed desirable to investigate the influence of profound dormancy in mammals, such as is characteristic of hibernating marmots, notwithstanding the fact that changes during lethargy in the Nissl granules, which are considered very labile, are doubtful (Rasmussen and Myers, '16). It was hoped that the enormous reduction in nearly all, if not all, vital processes of such animals during winter-sleep would add one more link to the chain of evidence for or against some of the theories concerning the connection between mitochondria and functional activity. Since alterations in the nervous system are important factors in all the more thoroughgoing hypotheses concerning the cause of hibernation (Rasmussen, '16 a), the facts discovered would be of interest also in connection with the mechanism involved in the production of dormancy.

MATERIALS AND METHODS

In this investigation there are involved fifteen adult woodchucks of the lot used in connection with a determination of blood volume as already reported (Rasmussen and Rasmussen, '17). The conditions under which the animals hibernated is there described. Five animals (two females and three males) were sacrificed between December 1st and December 3rd, just before the onset of hibernation, while still active and under full feed. Five others (two females and three males) were killed while dormant during the last few weeks of hibernation (between February 26th and March 18th). The remaining five (two females and three males) were bled between April 3rd and April 18th while active and after having been awake from four days to three weeks, but without having available any food or water either during or after winter-sleep.

After the blood was washed out by gradual perfusion with oxygenated Locke's fluid, warmed or cooled to the body temperature, according to the technique employed by Dreyer and Ray ('11), the vessels were flushed out with physiological salt solution and then Regaud's fixer (one part of commercial formalin neutralized with magnesium carbonate and four parts of a 3 per cent aq. sol. potassium bichromate) was allowed to perfuse the entire animal for an hour. In order to see if the length of the perfusion with Locke's solution had any noticeable effect on the mitochondria, the last animal of each of the three groups was perfused only long enough to cause a return of a colorless fluid from the veins before the fixer was injected, as is done regularly by Cowdry. These three control animals are at the bottom of each group in the accompanying table of results. Further treatment was done according to the outline given by E. V. Cowdry ('16 b). Sections were cut 2μ thick except in the case of the spinal cord where the sections were cut 3μ. Such thin sections were necessary to facilitate and make more accurate the counting of mitochondria.

At least four consecutive sections from a given block were mounted on each of ten or more slides and stained with a 20 per cent solution of acid fuchsin in aniline water and differentiated with a 1 per cent aq. sol. of methylgreen after various degrees of dechromation. Several slides were made under what appeared to be the optimum conditions so as to insure plenty of well-differentiated sections. By having four or more consecutive sections mounted on each slide it is possible to rule out any variation in thickness which might escape general inspection by counting the mitochondria in an equal number of cells from each of the four (or a larger even number) consecutive sections and using the average. As a matter of fact, only in a few cases was there any trouble in getting uniform cutting, despite the extreme hardness resulting from long fixation and the high melting point (60° to 62°C.) of the paraffin. A difference in thickness in sections cut as thin as 2μ is readily noticed by the much greater tendency of the thinner ones to wrinkle, so that only such regions of the ribbon as showed uniformity in cutting were used.

In selecting the levels for study important nuclei and ganglia in which the cells have a more or less well-known function were selected, as is seen in the accompanying table of results and related data. In the case of the spinal cord the sixth cervical segment was used for somatic motor cells of the ventral cornu and the seventh thoracic segment for visceral motor cells of the lateral cornu. The seventh thoracic and sixth cervical spinal ganglia were used for dorsal root ganglion cells. In the cerebellum the cortex from the inferior portion of the vermis was utilized. No comment is necessary on the other regions selected. It is believed that sufficiently varied types of cells have been chosen to be a fair index to the mitochondrial behavior in the nervous system under the conditions of hibernation and to rule out the objections frequently made to conclusions drawn from alterations detected in only a single type of cells (usually the Purkinje cells of the cerebellum) which often are apparently only remotely related to the physiological process under investigation.

In determining the number of mitochondria a Whipple eyepiece micrometer disc (Bausch & Lomb), on which squares of various sizes are ruled, was used. In general the medium-sized square ($\frac{1}{100}$ of the entire ruled field) was used as the unit. This area with the oil-immersion objective, ocular, tube length, etc., used and which were, of course, kept constant throughout the determinations, gave a field of $\frac{1}{13,611}$ sq. mm. In the case of sections 2μ thick the cubic volume represented by each square is $\frac{1}{6,805,500}$ cu. mm. In order to reduce all figures to the number of mitochondria in million per cu. mm. of cytoplasm the number of mitochondria found in the above small volume was multiplied by the factor 6.8. The corresponding factor for sections 3μ thick is 4.537. All figures given, therefore, indicate millions per cu. mm. of cytoplasm. For each type of cell in each animal the average of at least twenty fields from twenty different cells is given. Since there are five animals in each stage, the figure for each stage as a whole is the average of 100 cells.

As a preliminary study, the total number of mitochondria in such sections of cells as contained the nucleolus were counted. On account of the irregularity in shape and the great personal factor necessarily involved in deciding on what shall be considered the limits of the cell body, these figures are not given. They showed, however, exactly the same results as obtained by the quantitative determinations. Obviously, the results stated in terms of the number in a known volume of cytoplasm are the only facts capable of comparison with the work of other investigators. Due to the difficulty experienced, under the best optical conditions available, in determining the number of mitochondria when too closely packed, as they are occasionally in small clumps, the figures are necessarily only approximate; but they are believed to be sufficiently accurate for comparative purposes.

RESULTS

A description of the mitochondria of the nerve cells in this particular animal is unnecessary since they are essentially as described in other vertebrates by E. V. Cowdry ('12, '14), Busacca ('13), Nicholson ('16), and others. Suffice it to say that in the central nervous system they are usually granular near the nucleus and tend to become short rods more peripherally and long filamentous in the base of the processes and out in the dendrites and axon proper. In the spinal ganglion they are glandular or very short rods rather uniformly distributed throughout the cell body except in places where there is an accumulation of lipoid. In such regions the mitochondria tend to be excluded. This reciprocal relation between lipoid and mitochondria in spinal ganglion cells has been noted especially by Cowdry ('14) in a number of species of vertebrates. In no case, however, was there much lipoid, and in spinal ganglia, where most often encountered, only two or three cells in a section through an entire ganglion would contain any obvious lipoid by the method employed. But since there are innumerable gradations between granules and filaments, it was not practicable to determine any variation in the different types of mitochondria upon any quantitative basis. General examination, however, indicated no variation in shape or size characteristic of either particular individuals or of any of the three stages.

Many of what appeared to be rods are undoubtedly merely rows of two or more granules so closely packed that it is not possible to distinguish the separate components with the available apparatus. In imperfectly fixed tissue mitochondria are frequently clumped into larger masses which are clearly not representative of the normal condition. To what extent this occurs in the case of the best fixation is difficult to say. The long filaments in the axon and dendrites and in the cell body at the base of these processes are undoubtedly continuous filaments, since they are never seen as rows of granules and are always elongated continuous bodies even in the most superficial fibers in a block and next to large vessels where the fixation has been instantaneous while the tissue was still warm. Too much reliance cannot be placed on the form of mitochondria, at least within certain wide limits and especially in regard to length, for in living cells, as was observed by the Lewises ('15), elongated ones broke up into granules and granules fused into rods and they seemed to readily bend and assume various shapes as they moved about in the cytoplasm. E. V. Cowdry ('18) has pointed out that the form of mitochondria is not correlated with protoplasmic activity or quiescence.

The long perfusion with oxygenated Locke's solution (the maximum total length being one hour) at body temperature produced no obvious effect on the morphology or distribution of the mitochondria in nerve cells. Autolytic changes after death being comparatively slow in nervous tissue, no change should be expected. In fact, E. V. Cowdry ('18, p. 138) makes the statement that it is not even necessary to fix nervous tissue while still warm; "six or eight hours after death is often soon enough." It was, however, necessary to rule out this variable.

In regard to the number of mitochondria, the accompanying table gives the results in a compact form. It is clearly evident that the number of mitochondria, as already noted by Thurlow ('17), in a unit of cytoplasm in the cells of a given nucleus is comparatively uniform. The individual variations between neighboring cells of the same type was, of course, much greater than the averages tabulated. This is due, to a large extent, to the necessity of using such a small surface area—one that will fit in between the nucleus and the periphery of the cell. If the Nissl granules are large the mitochondria are less uniformly distributed in such a small square because in general the mitochondria lie between the masses of tigroid substance. The results to be of value must be based upon a sufficiently large number of fields to eliminate this irregularity of distribution. To do this in connection with the motor cells of the ventral horn it was necessary to count the mitochondria in at least twice as many unit areas as was used in general.

The only other attempt at quantitative determination of mitochondria is that by Thurlow ('17). This was done on the nuclei of the cranial nerves of the white mouse by utilizing sections 4μ

DATE	ANIMAL			NUMBER OF MITOCHONDRIA IN MILLIONS PER CUBIC MILLIMETER OF CYTOPLASM							
	Number	Sex	Rectal temperature	Spinal ganglion cells	Motor cells ventral horn	Motor cells lateral horn	Cell of Nuc. Grac. Nuc. Cun.	Purkinje cells cerebel.	Cells of superior coll	Betz cells motor cortex	Mitral cells olf. bulb.
Before hibernation. Active and fed											
			°C.								
December 1...	1–SIV	♂	35	300	185	252	253	360	229	326	261
December 2...	2–SIV	♀	36	305	174	260	266	349	335	300	260
December 2...	3–SIV	♂	36	293	181	258	254	362	246	307	246
December 3...	4–SIV	♀	36	309	195	255	261	354	244	295	259
December 3...	5–SIV	♂	35	287	178	259	242	351	236	297	262
Average				302	183	257	255	355	238	305	258
During hibernation. Dormant. Not fed since early in December											
February 26...	51–SIII	♀	7	298	180	256	254	251	240	300	265
March 3......	6–SIV	♀	15	295	198	250	240	344	249	296	255
March 4......	7–SIV	♂	16	310	189	258	258	359	232	305	262
March 17......	8–SIV	♂	14	286	187	246	255	341	236	303	259
March 18......	9–SIV	♂	12	293	194	266	249	355	243	302	265
Average				296	190	255	251	350	240	301	261
After hibernation. Awake. Active. Not fed since early in December											
April 3.......	56–SIII	♂	34	291	187	245	243	358	244	292	249
April 14.......	10–SIV	♂	34	312	177	267	260	348	220	290	266
April 14.......	11–SIV	♀	34	300	188	249	248	363	237	310	255
April 15.......	13–SIV	♀	36	312	186	260	258	355	240	299	258
April 18.......	15–SIV	♂	37	283	194	251	239	359	238	287	253
Average				300	186	254	250	357	236	296	256
Average of all animals				299	186	255	252	354	238	301	258

thick. Her results showed a variation in the number of mitochondria per cu. mm. of cytoplasm from 178 million (in the dorsal motor nucleus of the vagus) to 284 million (in the mesencephalic nucleus of the trigeminus). The final averages of the fifteen woodchucks will be seen to vary between somewhat higher limits or 186 million (in the motor cells of the ventral horn of the spinal cord) and 354 million (in the Purkinje cells of the cerebellum). The levels selected for this investigation and the small pieces of tissue taken from these levels did not include the nuclei examined by Thurlow, so that specific comparisons can not rigidly be made. The magnitudes are seen, however, to be of the same order, except that the upper limit is considerably higher. The number of mitochondria in the large motor cells in the nucleus of the hypoglossus of the white rat was among the lowest determinations (187 million) and agrees strikingly with the number here reported in the large motor cells of the spinal cord in the woodchuck, which is also the lowest determination (186 million). As was found by Thurlow, sensory cells are not distinguishable as a class from motor cells upon the basis of the number of mitochondria.

As has been observed before, particularly by E. V. Cowdry, now and then an individual cell will contain many more or, less frequently, distinctly fewer mitochondria than the neighboring cells of the same type, which, being found in the same region of the same section, must have been through exactly the same technique. This possibly indicates that individual cells may be in quite a different condition from the vast majority. Such a situation has been assumed to explain the classical Golgi technique when, as frequently happens, only here and there a cell is picked out and hundreds of surrounding cells are left unstained. In the spinal ganglion the few cells which contained an unusually large number of mitochondria were usually of the smallest type.

This more or less specific mitochondria-cytoplasmic ratio is another argument against the view maintained by Portier (see discussion in Compt. Rend. Soc. Biol., 1919, T. 82, pp. 244, 309, 337) to the effect that mitochondria are organisms living in symbiosis in larger cells.

The relationship of the mitochondria to the Nissl bodies as it appears in this investigation does not support the idea that the tigroid substance is normally more or less diffused throughout the cytoplasm and that the appearance of rather definite masses is an artifact produced by the reagents. Were this contention correct one would expect more of the mitochondria to be embedded in these precipitation products.

It further appears certain that there is no appreciable modification in the number of mitochondria as a result of the alterations attending hibernation, awakening, and subsequent inanition, thus testifying to the stability of these bodies under greatly modified functional conditions.

In the first place, during winter-sleep there is a great reduction in the metabolic processes. From the excellent summary of the literature on respiratory exchange during hibernation by Krogh ('16), it would appear that in mammals with a normal body temperature of about 36°C. when awake and whose body temperature approaches 10°C. or less during hibernation, the oxygen consumption falls to one-twentieth or less of the amount used before dormancy occurred. The CO_2 eliminated decreases relatively much more. This great reduction in oxidation processes in the body does not apparently affect the mitochondrial content of nerve cells, although in all probability the nervous system shares at least to some extent in the reduced oxygen consumption. There is, then, from this source no evidence in favor of the theory that mitochondria are associated with oxidation processes. The possibility exists, however, that they may be involved in such processes without showing any morphological or numerical changes with degrees of activity. N. H. Cowdry ('18) found in myxomycetes that the mitochondria were found in all stages of the organism, even in fully formed spores with a thick horny capsule and supposedly in a state where the physiological processes are nearly at a standstill.

What other tissues in the woodchuck will show remains to be determined. The glands are now under investigation.

During hibernation the absorbing power of the blood for CO_2 decreases and there is a distinct increase in the amount of CO_2 actually found in the blood (Rasmussen, '16 b). These changes as found in the venous blood reflect an increase in the H-ion concentration of the tissues. This tendency toward acidosis does not seem to have any effect on the mitochondria in nerve cells, although there must be readjustments in the nervous system to this altered condition of its blood supply.

Next we may mention the relation of mitochondria in nerve cells to the transition of a mammal from the warm-blooded (homoiothermal) type with a body temperature of about 36°C. to what is in many respects the cold-blooded (poikilothermal) type with a temperature only slightly higher than that of the surroundings, and which reached as low as 7°C. in one animal here involved. This striking alteration seems to have had no effect.

Finally, attention is drawn to the fact that during dormancy and for as long as three weeks after waking up, i.e., until the last animal was killed, no food or water was available. This inanition during hibernation as well as after becoming active did not apparently affect the mitochondria of nerve cells, although during the three months of winter-sleep the body weight decreases about one-fourth while the animals allowed to live several weeks after waking up lost fully one-third.

SUMMARY

1. Profound dormancy such as is seen in a fully hibernating marmot with a rectal temperature as low as 7°C. does not affect noticeably in any way the mitochondria of the central nervous system or of the spinal ganglion.
2. Complete inanition for three months during winter-sleep and for three weeks after waking up does not modify the morphology, number, or distribution of mitochondria in nerve cells.
3. Perfusion with oxygenated Locke's solution at body temperature for a period as long as one hour does not modify the mitochondrial content of nerve cells beyond what is produced by a more rapid flushing out of the vessels for a duration of only fifteen minutes.

BIBLIOGRAPHY

Busacca, A. 1913 L'apparato mitocondriale nelle cellule nervose adulte Arch. f. Zellforsch., Bd. 11, S. 327–339.

1915 Sulle modificazioni dei plastosomi nelle cellule dell'epitelio pigmentato della retina sotto l'azione della luce e dell' oscurità. Ricerche Lab. Anat. Univ. Roma, vol. 18, pp. 217–237.

Cowdry, E. V. 1912 The relation of mitochondria and other cytoplasmic constituents in spinal ganglion cells of the pigeon. Intern. Monatsschr. f. Anat. u. Physiol., Bd. 29, S. 473–504.

1914 The comparative distribution of mitochondria in spinal ganglion cells of vertebrates. Am. Jour. Anat., vol. 17, pp. 1–29.

1916 a The general functional significance of mitochondria. Am. Jour. Anat., vol. 19, pp. 423–446.

1916 b The structure of chromophile cells of the nervous system. Contributions to Embryology, vol. 4 (Publication no. 224, Carnegie Institution, Washington), no. 11, pp. 27–43.

1918 The mitochondrial constituents of protoplasm. Contributions to Embryology, vol. 8 (Publication no. 271, Carnegie Institution of Washington), no. 25, pp. 39–148.

Cowdry, N. H. 1917 A comparison of mitochondria in plant and animal cells. Biol. Bull., vol. 33, pp. 196–228.

1918 The cytology of the myxomycetes with special reference to mitochondria. Biol. Bull., vol. 35. pp. 71–94.

Dreyer, G., and Ray, W. 1911 The blood volume of mammals as determined by experiments upon rabbits, guinea-pigs and mice; and its relationship to the body weight and to the surface area expressed in a formula. Phil. Trans., London, Series B, vol. 201, pp. 133–160.

Duesberg, J. 1919 On the present status of the chondriosome-problem. Biol. Bull., vol. 36. pp. 71–81.

Krogh, August 1916 The respiratory exchange of animals and man. Longman Green & Co., pp. 124–130.

Levi, G. 1896 Contributo alla fisiologia della cellula nervosa. Riv. di Patol. Nerv. e Ment., vol. 1, pp. 168–180.

Lewis, M. R., and Lewis, W. H. 1915 Mitochondria in tissue cultures. Am. Jour. Anat., vol. 17, pp. 339–401.

Luna, E. 1913 Sulle modificazioni dei plastosomi delle cellule nervose nel trapianto ed in seguito al taglio dei nervi. Anat. Anz., Bd. 44, S. 413–415.

M Cann, Gertrude F. 1918 A study of mitochondria in experimental poliomyelitis. Jour. Exper. Med., vol. 27, pp. 31–36.

Motta-Coco, A., e Lombardo, G. 1903 Contributo allo studio della cellula dei gangli spinali. Anat. Anz., Bd. 23, S. 635–640.

Nicholson, N. C. 1916 Morphological and microchemical variations in mitochondria in nerve cells of the central nervous system. Am. Jour. Anat., vol. 20, pp. 329–349.

Rasmussen, A. T. 1916 a Theories of hibernation. Am. Naturalist, vo . 50, pp. 609–625.

Rasmussen, A. T. 1916 b A further study of the blood gasses during hibernation in the woodchuck (Marmota monax)—The respiratory capacity of the blood. Am. Jour. Physiol., vol. 41, pp. 162–172.

Rasmussen, A. T., and Myers, J. A. 1916 Absence of chromatolytic change in the central nervous system of the woodchuck (Marmota monax) during hibernation. Jour. Comp. Neur., vol. 26, pp. 391–401.

Rasmussen, A. T., and Rasmussen, G. B. 1917 The volume of the blood during hibernation and other periods of the year in the woodchuck (Marmota monax). Am. Jour. Physiol., vol. 44, pp. 132–148.

Scott, F. H. 1905 On the metabolism and action of nerve cells. Brain, vol. 28, pp. 506–526.

Strongman, B. T. 1917 A preliminary experimental study on the relation between mitochondria and discharge of nervous activity. Anat. Rec., vol. 12, pp. 167–171.

Thurlow, M. DeG. 1917 Quantitative studies on mitochondria in nerve cells. Contribution to Embryology, vol. 6 (Publication no. 226, Carnegie Institution of Washington), no. 16, pp. 35–44.

351

No. 14

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MAY 1

Reprinted from JOURNAL OF MORPHOLOGY,
Vol. 32, No. 2, June, 1919

THE DEVELOPMENT OF THE GASTRIC GLANDS IN SQUALUS ACANTHIAS

ADOLPH R. RINGOEN

Department of Animal Biology, and the Institute of Anatomy, University of Minnesota

SEVEN FIGURES (THREE PLATES)

CONTENTS

Introduction 351
Literature 352
Observations 353
  A. Early changes in the gastric epithelium 353
  B. Formation of definite gland rudiments 355
  C. Influence of the gland rudiment on the epithelium 356
  D. Subsequent history of the gland rudiments 357
  E. Rotation of cells 359
  F. Histology of the fully differentiated gastric gland 362
Discussion of results and literature 363
Summary 368
Bibliography 370

INTRODUCTION

The digestive tract of fishes, although the subject of a considerable amount of research, still affords opportunity for further study. The very early investigators of the subject were interested in its glands, but no serious attempts were made to determine their origin and further differentiation. A study of the literature shows that there is still comparatively little written on the histogenesis of these structures. In view of these facts, the present paper attempts to elucidate the sequence of events that takes place in the development of the gastric glands as found in Squalus acanthias.

The material used in this study was placed at my disposal by the Department of Anatomy, University of Minnesota. For the study of the glands, the stomach was removed from the specimen, embedded in paraffin, and cut in serial sections 5 μ in thickness. The sections were stained with iron-haematoxylin. Both erythrosin and orange G were employed as counterstains. For a large part of the prepared Acanthias material I am personally indebted to Dr. Richard E. Scammon, who has so kindly permitted me to use numerous series from his private collection (S. C.).

It is with great pleasure that I express my appreciation to Dr. Scammon for suggesting this investigation, for the loan of material, and for the interest shown during the progress of this study. I also wish to express my indebtedness to Mrs. Helen Sanborn Chapman for the accurate drawings.

LITERATURE

Our conceptions of the histogenesis of the glandular elements of the digestive tract are based largely on observations made on the study of mammalian material. A comprehensive review of the literature bearing on the subject would be foreign to the purpose of this study. Later an attempt will be made to indicate the present status of our knowledge in this field, in so far only as it may be essential to a clearer understanding of gland formation in Squalus acanthias, by brief reference to a few papers.

Sprott Boyd ('36) made the first observations on the presence of gastric glands in mammals and fishes. Following Boyd's observation, Bischoff ('38) studied the mucous lining of a great many species of fishes. In some species he was unable to find glands, while in others they were abundant.

In 1852 Leydig discovered gastric glands in Squatina angelus and Torpedo galvani. Later he referred to these glands as 'Labdrüsen,' thinking that they were comparable to the gastric glands of mammals.

Edinger ('77), in studying the mucous membrane of the stomach of fishes, was unable to distinguish the chief and central cells as discovered by Heidenhain and Rollet in the mammalian stomach. According to Edinger, among the Teleosts there are a number of forms that possess no glands in the stomach.

He believes that the gastric glands appear phylogenetically first in the selachians.

The literature on the glands of the Selachii has been to a large extent reviewed by Oppel ('96), but one finds very little information relative to the origin of the gastric glands themselves.

Sullivan ('07), in the course of a study devoted to the digestive tract of Elasmobranchs, considers primarily the physiological features of the glands. He makes no comment upon the development of the glands.

Peterson ('09) is, as far as I am able to ascertain, one of the few investigators to consider in some detail the development of the gastric glands in selachians. In his studies on the histogenesis of the glands in a number of these forms he describes and figures epithelial outgrowths as the rudiments of glands.

OBSERVATIONS

In Squalus acanthias, gland development proceeds in a very different manner from that commonly described for a number of mammals, including man. The conditions in the gastric epithelium of embryos 133 mm. in length indicate that in the selachians gland formation is not associated with the formation of gastric pits, as has been claimed for man. Since Acanthias specimens of this length show the first traces of gland differentiation, I begin with this stage and follow out in later stages the complete evolution of a gland.

A. Early changes in the gastric epithelium

The early stages in the development of gastric glands are clearly followed in Squalus acanthias embryos 133 mm. long. In fact, the very beginnings of the glands are discernible here as differentiating in the gastric epithelium itself. At this particular stage the epithelium of the stomach is characterized by its great activity in the way of undergoing definite local changes throughout its entire extent. Prior to the 133-mm. stage there were no apparent variations or irregularities in it;

all of its constituent cells presented similar morphological features and identical staining reactions. The nuclei also presented their own characteristic configuration and staining reactions. With the establishment, however, of local changes in the gastric epithelium (133-mm. embryos) there appear certain definite modifications in its cellular make up. Figure 2, from a 133-mm. embryo, shows a number of these modifications. The two cells on the extreme right and left, respectively, of this figure represent the typical columnar epithelial cells so characteristic of the selachian stomach. They are long and narrow, and are without a basement membrane. The cytoplasm stains a faint grayish tint with iron haematoxylin-orange G. The nuclei are long and slender; they present a deeper gray tint than does the cellular cytoplasm. The four cells just described are destined to remain as epithelial cells. Interpolated between, however, are a number of other cells which are characteristically different from the former in both their cytoplasmic and nuclear staining reactions. A number of these cells may still show a close relationship to the neighboring epithelial cells as exemplified by their behavior toward the iron haematoxylin-orange G stain. In most cases, however, the cytoplasm and the neuclus do not stain in the same manner as in the adjacent epithelial cells. The whole tendency of staining variations in this direction is for an epithelial cell to decrease in its avidity for the stain as soon as it is called upon to assume a different rôle from that of an ordinary epithelial cell. At times such a change in the staining reactions of the cell body may precede somewhat that of corresponding changes in the nucleus or vice versa, and again the changes may proceed rather synchronously in both the cytoplasm and the nucleus.

In addition to the above-described staining reactions, there is also a further change in the form of the nucleus, as shown in figure 2. On the right-hand side of the figure, in the third cell from the margin, is a nucleus which has not changed its staining reactions, although the cell body has progressed to some extent in that direction. It is apparent from the figure that the nucleus is undergoing a change in its shape. No longer does it correspond in its configuration to the long narrow epithelial nucleus contained in the cells at the extreme right and left of the figure. A decided change in shape is clearly seen in those cells which have changed both their cytoplasmic and nuclear staining reactions.

If the conditions in the gastric epithelium of 133-mm. Acanthias embryos, as I have described them in figure 2, are interpreted properly, they would indicate that in this group gland development is of a rather primitive nature. No specialized cells are set apart in early embryonic life for the origin of glands, but the general epithelium is endowed with the capacity of transforming certain patches of its constituent cells into definite gland rudiments at the proper time. When the proper time is at hand, small groups of these apparently similar epithelial cells change their staining reactions and the shape of their nuclei, and differentiate in another direction. They continue to evolve in a very definite direction, because they are incapable of giving rise to any other structure than the particular gland rudiment towards which their potentialities are directed.

B. Formation of definite gland rudiments

The characteristic changes which appear in the gastric epithelium at the 133-mm. stage are even more pronounced in slightly longer embryos. In 137-mm. specimens the epithelium has been modified only at those points where glands are to be formed. It is surprising with what regularity the apportioning of the general epithelium into glandular and nonglandular areas has taken place at this stage. The number of epithelial cells which intervene between two potential gland areas is practically the same in all cases. Just what factors determine the selection of certain groups of epithelial cells as the precursors of glands to the absolute exclusion of others obviously can no more be answered than why certain entodermal cells will differentiate into liver cells, while other similar cells, at least so in their early stages of differentiation, will give origin to pancreatic tissue.

On comparing figures 2 and 3 (the latter an embryo of 137 mm.), it is apparent that gland rudiments are now sharply marked off from the neighboring epithelial cells. In embryos of 137 mm. in length, such areas are very numerous. The entire epithelium of the stomach is literally studded at regular intervals with them. At this time none of the cells making up a gland rudiment present staining characters bordering on those of the epithelial cells. The nuclei present about the same staining reactions as portrayed in figure 2. There has been, however, a considerable progressive change in their shape.

Figure 3 shows two gland rudiments embedded in the epithelium. They present such a striking appearance in sections of the stomach (137-mm. specimens) that one cannot fail to notice them. The regularity in their distribution is indeed striking. Never have I seen similar rudiments lying at the base of the epithelium. Obviously, there would be no reason for such a location, since the early gland rudiments are simply transformed epithelial cells. In the same figure the two gland cells at the extreme right represent only a portion of a gland rudiment, due to the plane of sectioning.

That the potentialities of the epithelial cells are by no means the same is particularly evident in the gastric epithelium of Acanthias specimens 137 mm. in length. Every gland of the adult specimen is represented at this stage by its own epithelial modification or gland rudiment.

C. Influence of the gland rudiments on the epithelium

The differentiation and presence of the gland rudiments in the epithelium has had a profound influence on the final configuration of the epithelium itself. During the early stages of differentiation it increases in thickness; at no time is it stratified, although in the early stages of embryonic development the disposition of the nuclei in several planes simulates stratification. In Acanthias embryos 133 mm. in length, the epithelium consists of columnar cells with their lateral surfaces closely approximated (fig. 2). The differentiation and presence of gland rudiments have changed this simple uniform condition. No longer are the epithelial cells arranged in the form of a single continuous row in embryos 137 mm. long. On comparing figures 2 and 3, it is evident that as soon as a gland rudiment is well marked out in the epithelium (fig. 3) it forms a bottle-like plug. The lower expanded portion of the plug tends to press upon the neighboring epithelial cells, thus crowding them closer and closer together. This lateral displacement of the epithelial cells proceeds unhampered, as they are not anchored fast by a basement membrane, but may extend freely down into the underlying mesodermic tissue.[1] As a result of compression exerted by the expanded part of the bottle-like plug of the gland rudiment, or for a lack of space, the epithelial cells are closely approximated at their bases. Since the distal parts of the epithelial cells have not been affected by the mechanical forces involved in the compression phenomena, it naturally follows that the portion of epithelium intervening between two gland rudiments takes on a fan-shaped form in cross-section (fig. 3). This peculiar arrangement of the epithelial cells, as compared with the simple uniform condition in the earlier stages, is maintained in the later stages.

D. Subsequent history of the gland rudiments

The subsequent history of the gland rudiments will now be considered in detail. Acanthias embryos 137 mm. long furnish very favorable material for such considerations. At this stage many of the gland rudiments are undergoing a great change in their length; their constituent cells are growing out into the mesodermic tissue. During the course of a very short period of time every gland rudiment will have elongated and burrowed its way into the underlying tissue.

Figure 4 represents one of the so-called epithelial outgrowths in the gastric epithelium of an Acanthias embryo 137 mm.

---

[1] Hopkins ('95) believes that a basement membrane does not exist in the ganoids. According to Edinger ('77), fishes possess no basement membrane, but the epithelium borders directly upon the underlying tissue.

long. At this stage the constituent cells of a gland rudiment, and as illustrated in figure 3, have simply elongated and are pushing their way out into the underlying mesodermic tissue. During the pushing and burrowing of an outgrowth down into the mesodermic tissue there is an actual migration of its cells. Now the nuclei have increased greatly in size, as compared with those shown in the gland rudiment of figure 3, and at this stage they tend to occupy the most distal parts of the outgrowth. No doubt the increase in the size of the nuclei at this time—preparatory to division—is associated with the fact that the cells which contain them are to grow out still further at a subsequent time, and obviously this requires additional cells (see fig. 5, also from a specimen 137 mm. long, for documentary evidence on the further growth of a gland rudiment; increase in the number of nuclei with a distinct change in their size and shape, as compared with fig. 4). Cell outlines are just as distinct here as they were before the gland rudiment cells began to grow out into the mesodermic tissue (compare fig. 2 with fig. 3).

In slightly more advanced stages of differentiation than is represented in figure 4, an outgrowth becomes more or less tubular in form. This condition is represented in figure 5, also from a 137-mm. specimen. With still further differentiation, as I shall attempt to show at a subsequent time, this simple condition is radically changed.

Another point of considerable interest in connection with figure 5 is the position of the nuclei in the tubular outgrowth. Those located in the region where the gland rudiment first burrowed do not seem to present any definite arrangement in their distribution within the outgrowth. On the other hand, most of the nuclei which are found in the lower half of the outgrowth show that their long axes are placed parallel to the long axes of the tube. In slightly more differentiated outgrowths this parallel arrangement is decidely modified. Even as shown in figure 5, there is a slight tendency in this direction, for several of the nuclei are on the verge of shifting their axes.[2]

---

[2] The nuclei, as will be pointed out later, are not actually changing their long axes, but the cells containing them are shifting.

During the extension of a glandular outgrowth down into the mesodermic tissue it meets with a small amount of resistance for two reasons. In the first place, the constituent cells of an outgrowth are not anchored fast by any structure comparable to a basement membrane, and in the second place, the mesodermic tissue is of such a loose character that it is simply pushed back and more or less condensed by the burrowing cells (figs. 4, 5, 6, and 7).

E. Rotation of cells

Figure 6, from an embryo 137 mm. long, shows a glandular outgrowth at its maximum length. On comparison with figure 5, it is apparent that the staining reactions of both the nuclei and cytoplasm are identical. The morphological features of the nuclei are also the same. The glandular outgrowth is no longer a tubular structure; the shape of its proximal end has been characteristically modified. This change of form may be ascribed to a rotation of cells in the lower two-thirds of the outgrowth. Although cell boundaries are not distinguishable in this figure, it is apparent on comparison with figure 5, in which instance cell boundaries are also absent, that in following the course of the nuclei in figure 6, as compared with those shown in figure 5, one is simply tracing out the movements of the cells that contain them. A number of cells, as represented in figure 6, have rotated through an angle of about 90°. The completion of the rotation process is seen in figure 7 (from a specimen 146 mm. long). In this stage cell boundaries are evident; the long axes of the nuclei, instead of being placed parallel to the long axes of the future gland, as they were in the tubular outgrowth represented in figure 5, are now placed at right angles to the lumen of the gland. Scammon ('15), in the course of a study devoted particularly to the histogenesis of the selachian liver, describes a similar rotation of cells in tubule anastomosis. He does not think that the nuclei shift their axes within the cells, for he frequently finds, in cases where faint cell boundaries can be made out, that the cells show the same changes in position as do the nuclei.

In order to elucidate more clearly the sequence of events that takes place in the evolution of a selachian gland from the simple tubular condition on through to the typical flask-shaped form, it has seemed feasible to submit a number of diagrams illustrative of the rotation processes. A casual reference to these diagrams will enable one to comprehend at a glance, without the aid of detailed figures and descriptions, that an actual rotation of cells play a fundamental rôle in molding the fundic portion of the Selachian gastric gland.

In figure 1, diagram A, the glandular outgrowth has extended only a short distance, and in most instances the long axes of the nuclei are parallel to the long axes of the outgrowth. This is the condition one would expect to find, since in a slightly younger stage (fig. 3) the nuclei are also parallel to the long axes of the gland rudiment. Diagram 1B shows that the glandular outgrowth has not only increased in length, but that a number of the nuclei are beginning to change their long axes with reference to the long axes of the outgrowth. With reference to these shifting movements Scammon ('15) states "that it is hardly to be considered that the nuclei shift their axes within the cells." Since cell boundaries were not seen in the specimen, illustrated in figure 1B, it may be reasonably assumed that the shifting movements of the nuclei are but the shifting movements of the cells which contain them. The next diagram (fig. 1C) distinctly shows that the cells in the region of what is to form the future fundic portion of the gland have rotated through an angle of about 90°. It is very probable that the cells located at what may be called the center of the base of the fundus do not change their positions, since their nuclei show no shifting movements at any time. Diagram 1D shows that all of the nuclei have passed through a change in their original axes of about 90°. The long axes of the nuclei now lie at right angles to the lumen of the gland. In figure 7 the outlines of the cells were drawn just as they appeared in the actual specimen. This shows that the rotation of the nuclei is to be interpreted only as the shifting movements of the cells that contain them.

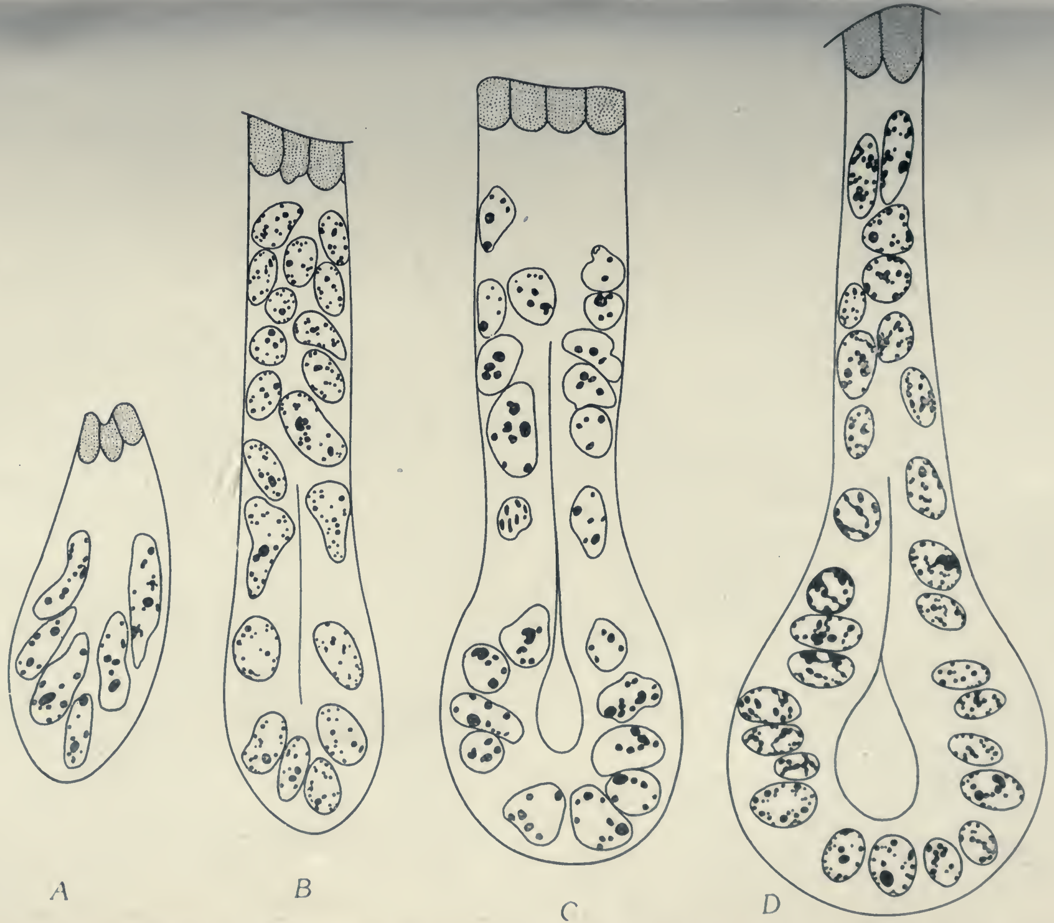


Fig. 1 Diagrams showing four stages in the evolution of a selachian gastric gland. All of these diagrams are drawn from actual specimens. All of the nuclei were outlined with the camera lucida and drawn with the same magnification, × 800. 1A is drawn from an embryo 133 mm. in length; 1B and 1C from embryos 137 mm. in length, and 1D from a 146-mm. specimen.

F. Histology of the fully differentiated gastric gland

Figure 7 (embryo 146 mm. long) represents the fully differentiated gastric gland. In specimens of this length the vast majority of glands present such an appearance, although there may be slight variations in the length of the neck and in the size of the fundic portion. These slight variations are, however, of no fundamental importance, for they do not signify that the glands differ in character.

All of the gastric glands are unbranched, flask-shape structures. Never have I been able to find the bifurcated forms described by Peterson('09). He believes that the neck cells of the gland are of a rather primitive character during their early history, and that they may give origin to a side bud which with further differentiation, gives to the gland a forked appearance.

At no time have I been able to distinguish more than one type of cell in a gland (fig. 7). All authors who have investigated the gland cells of lower vertebrates are quite agreed in the occurrence of a single cell-type.[3] According to Edinger ('77), in the fishes, this single type is homologous neither to the chief nor to the parietal cell of the mammalian stomach. His conclusion has been generally accepted. It appears from the investigations of numerous other observers that the differentiation of two the cell-types in the gastric glands of fishes does not obtain. This specialization probably appears phylogenetcally much later.

As far as Squalus acanthias is concerned, and as Peterson ('09) maintains for a number of selachians, the neck cells of a gastric gland are not concerned in the formation of any specific secretion. This activity appears to be confined to the cells making up the fundic portion of the gland. In all the cases that have come under my observation, I have found that the secretion granules are elaborated exclusively in these cells. As to the precise character of these granules, I have no definite knowledge.

---

[3] Oppel ('96) agrees with Edinger in the finding of only one type of cell in the glands of fishes, but states that this single type appears to possess relationships with the parietal cell of mammals.

DISCUSSION OF RESULTS AND LITERATURE

Miss Ross ('02), in her investigations on the development of the gastric glands in Desmognathus, Amblystoma, and pig, finds that the round granular cells which give origin to the glands appear as distinct cells with the differentiation of the entoderm. These gland cells occur at the base of the epithelium in close contact with the basement membrane.

In addition to Miss Ross ('02), Toldt ('81) also maintains that the beginnings of the gastric glands in the cat are represented by granular cells, which are interpolated at the basal parts of the epithelial cells. There are, however, numerous other investigators who ascribe the origin of glands to epithelial projections, or outgrowths,[4] among whom may be mentioned Kölliker ('52), Brand ('78), Griffini and Vassale ('88), Oppel ('96), Minot ('02), and Peterson ('09). Johnson ('10) finds in a human embryo of 120 mm. that the beginnings of the gastric glands appear as knob-like outgrowths at the bottoms of the gastric pits. It is generally admitted by those who believe that the glands arise from downgrowths of the surface epithelium, that the gastric pits—later elongated to form grooves—give origin to the glands in this manner.

In specimens of Squalus acanthias there are no gastric pits; the longitudinal folds, which are presumably the forerunners of the villi in higher forms, are variable in their number, location, and size. In all probability, their presence is due to the contraction of the muscular coats. Consequently, gland development must go on in a very different manner from those forms where all these features can be readily identified. Peterson ('09) states that in a number of selachians he has found that they arise from epithelial outgrowths directly. With regard to the origin of the outgrowths themselves, and their further history as based more particularly on a study of Acanthias vulgaris, he states:

---

[4] Bensley ('00) finds in a Urodele larva 11 mm. in length that the oesophageal glands also appear as tubular downgrowths of the foregut entoderm. He believes that these glands represent gastric glands whose development has been arrested.

> . . . . es erreichen nicht alle Zellen die freie Oberfläche, sondern viele bilden eine untere Schicht und entbehren des Pfropfes. Diese lassen die Magendrüsenzellen aus sich hervorgehen. Sie wandern aus, ein kurzer, halbkugeliger Fortsatz ragt aus dem Epithel heraus, vergrössert sich, der Kern rückt nach. Andere dicht daneben liegende tun dasselbe, so dass eine Knospe an der Epithelbasis zum Vorschein kommt. Die Zellen bleiben mit einem lang ausgezogenen Ende zunächst noch mit dem übrigen Epithel in Verbindung. Immer mehr Zellen rücken nach und drängen die erst ausgewanderten weiter . . . .

Since I have already discussed the origin of gland rudiments in Squalus acanthias specimens, no further attempt will be made here to consider them in detail. Suffice it to say that in specimens 133 mm. long, gland rudiments differentiate in the gastric epithelium itself—not from special embryonic cells set apart with the early differentiation of the entoderm (as Miss Ross ('02) maintains for Desmognathus, Amblystoma, and pig), —but from typical epithelial cells (fig. 2).

It may be remarked that Peterson's observation that in Acanthias vulgaris embryos of 55 to 70 mm. in length not all of the epithelial cells reach to the free surface—lumen side of the stomach—"sondern viele bilden eine untere Schicht und entbehren des Pfropfes. Diese lassen die Magendrüsenzellen aus sich hervorgehen . . . ," is not in accordance with what I find in the epithelium of Squalus acanthias. For it is clearly seen in embryos of less than 133 mm. in length that all of the epithelial cells at this stage present the closest morphological features and staining reactions. They are all of equal length, and, therefore, they all reach to the surface.[5] Even at the 133-mm. stage, and as shown in figure 2, all of the epithelial cells still reach to the surface. There are, however, considerable changes in the staining reactions of small groups of them. These changes are very significant, for they show that, in spite of their morphological similarities, not all of the epithelial cells are endowed with the same specificities for further differentiation. Small groups of them will remain as epithelial cells, while other groups change their staining reactions, and the shape of their nuclei, and finally form a bottle-like plug which is embedded in the epithelium (fig. 3). All of these changes were considered in detail in connection with figures 2 and 3.

---

[5] In support of my results I wish to quote from Kirk's ('10) paper with particular reference to Toldt's ('81) work on the rudiments of glands in the fundic portion of the cat's stomach as large, eosinophilic cells interpolated at the base of the surface epithelial cells. "Toldt is sure these cells are of epithelial origin, but believes they at no time reach the surface, being always shut off from the latter by the overhanging distal ends of the tall pyramidal surface epithelium; he suspects that they arise from young Ersatzzellen. His Ersatzzellen have almost certainly been shown by the work of Stöhr (1882) and Bizzozero (1888) to be 'Wanderzellen.' Griffini and Vassale maintain that Toldt's figures and text harmonize remarkably with their findings, except that Toldt, through use of oblique sections, erroneously concluded that these primary gland cells do not reach the surface, and that their lumen is thus not at first continuous with the

Since Peterson maintains that the gastric glands grow out from portions of the epithelium whose cells do not reach the surface, it would be interesting to know how he would account for certain nuclear variations. In figure 5 the nuclei are numerous in the glandular outgrowth. On comparing one of them with the epithelial nucleus on the left-hand side of the same figure it is apparent that there are fundamental differences with reference to their form, and also in the amount and distribution of their chromatin material. These differences were also mentioned in connection with figures 2 and 3. How would Peterson explain such differences? As far as the nuclear differences in figures 2 and 3 are concerned, he woud be unable to give any logical explanation, because he failed to find similar stages in his material. Now, if the gastric glands in Selachians really arise by means of epithelial outgrowths, as Peterson holds, one would naturally expect to find similar nuclei in both the outgrowths and the general epithelium. Figures 4 and 5, however, show that this is not the case. They are very different in their form, size, and in their arrangement of chromatin material. This is precisely what one would anticipate after studying stages similar to the ones depicted in figures 2 and 3 of this paper.

stomach lumen. Griffini and Vassale found many such groups with lumina apparently shut in on all sides, but reconstructions always demonstrated continuity with the stomach lumen from the first."

With reference to the formation of the gland lumen, I find that it arises in the manner described by Peterson. The early formation of it is frequently seen as a slight indentation on the free surface of a well-defined gland rudiment, shortly after it has pushed out into the underlying mesodermic tissue. The further extension of the gland rudiment is followed by a similar extension of the gland lumen. As to whether the extension of the lumen into the fundic portion is responsible for the shifting movements of the cells, I am unable to say. At all events the extension of the lumen into the fundic portion of the glands does not cause the cells there to take on a 'schräge' position with reference to the lumen, as Peterson maintains I have already pointed out that in the fully differentiated gastric gland the cells at the fundic portion are placed at right angles to the lumen of the gland with reference to their long axes.

In spite of his observations relative to the origin of gastric glands in selachians, Peterson is at a loss to account for the great numbers of these structures that he finds in old specimens. To quote from his paper:

> Wie erfolgt nun die weitere Vermehrung der Drüsen? Bei einem jungen Tiere hat die Schleimhaut dasselbe Aussehen wie bei einem alten. Es kommen genau so viel Drüsen auf den Quadratmillimeter; auf einer Schnittstrecke von 1 mm. (Schnittdicke 10 μ) liegen quer- wie längsgeschnitten 20 Schnitte im Durchschnitt, also 400 auf den Quadratmillimeter. Ein Magen eines jungen Tieres (Magenlänge 5 cm.) habe n qmm Fläche, der eines alten (Magenlänge 10 cm.) sagen wir dann 4 n qmn Fläche, so kommen auf den einen Magen 400 n, auf den anderen 1600 n Drüsen, wo kommen diese 1200 n weiteren Drüsen her?

In order to account for these 1200 new glands Peterson thinks that there may be a longitudinal splitting of the young glands. As a result of this cleavage process there are now two glands where before there was only one. He also thinks that "Verzweigungen der Drüse in den mittleren Partien könnten als Anhaltspunkt dienen" for increasing the numbers of the glands. Furthermore, Peterson thinks that the neck cells of a young gland may contribute to the formation of additional glands: "Sie haben wir oben als indifferente Reste der alten Anlage kennen gelernt, und sie könnten also auch späterhin Drüsenknospen entwickeln. Die anderen Zellen der basalen Reihe sind vollkommen verschwunden—aufgebraucht." He states, however, that he has no proof for such activity on the part of the neck cells.

Peterson's statement, "bei einem jungen Tiere hat die Schleimhaut dasselbe Aussehen wei bei einem alten," is absolutely not in accordance with my observations. Figures 2 and 3 show that there are decided morphological and staining differences, and in these specimens, from which the drawings were made, there is only a difference of 4 mm. in length. In figure 2 (embryo 133-mm. long) the epithelium is undergoing definite changes, while in figure 3 (137-mm. long) it shows more decided changes—the rudiments of glands.

It is unnecessary to build any elaborate theories with reference to the manner in which the number of glands is increased, as Peterson has attempted to do. Any attempt with such an aim in view is diametrically opposed to what one finds in the gastric epithelium of Acanthias specimens 137 mm. in length. At this stage the epithelium has been modified at only those points where glands are to be established, and, indeed, it is surprising with what regularity and precision these changes occur. The entire epithelium is literally studded at regular intervals with them. Every gland of the adult animal is represented at this stage by its own epithelial modification, or gland rudiment. In specimens 146 mm. long and above this length, all the rudiments have given origin to glands, and the neck portion of every gland occupies the same relative position in the epithelium as did the early gland rudiment. The entire process of glandular development takes place in just as orderly a manner as does the differentiation and development of any other vertebrate organ or structure.

Peterson's entire difficulty in failing to be able to account for the vast numbers of glands in old specimens is easily explained. Although he saw numerous epithelial buds ('Knospen') in young specimens, they were by no means sufficiently numerous to account for all the glands in the adult specimen.

Undoubtedly the specimens that he examined showed comparatively few buds. I have also frequently found that in embryos 137 mm. in length they are not especially numerous. Therefore, since Peterson did not discover the precursors of the buds themselves, or the gland rudiments as I have called them throughout this paper, he is tempted to theorize with reference to the manner in which the number of glands is increased.

SUMMARY

1. In the selachians, as represented by Squalus acanthias, no specialized cells are set apart in early embryonic life for the formation of glands (as described by Miss Ross for Desmognathus, Amblystoma, and pig), but the general epithelium is endowed with the capacity of transforming certain groups of cells into definite gland rudiments.

2. The apportioning of the gastric epithelium into glandular and non-glandular areas is evident in Acanthias embryos 133 mm. in length. At this stage the epithelium is undergoing local modification in that small groups of its constituent cells change their staining reactions (iron haematoxylin-orange G) as compared with the adjacent epithelial cells (fig. 2). As soon as an epithelial cell is called upon to become a contributory member toward the formation of such a group of cells, both its cytoplasm and nucleus decrease in their avidity for the above-mentioned stain.

3. In addition to this change of staining reactions, there is a further change in the morphology of an epithelial nucleus. Many a nucleus is seen in the process of changing its shape from the typical narrow, elongated type, so characteristic of the young epithelial cell, to the plump nucleus of a gland rudiment cell.

4. That the potentialities of the epithelial cells are by no means the same is particularly evident in the gastric epithelium of Acanthias specimens 137 mm. in length. At this stage the epithelium is studded at regular intervals with well-defined groups of cells. These are no longer to be considered as epithelial cells, but the rudiments of glands (fig. 3).

5. The presence and further differentiation of the gland rudiments in the epithelium play an important part in causing the proximal free ends of the epithelial cells to be laterally compressed. As a result of compression, the epithelial cells intervening between two gland rudiments are forced to take on a fan-shaped arrangement in cross-section (fig. 3).

6. Acanthias embryos 137 mm. in length show that many of the gland rudiments are growing out into the underlying mesodermic tissue to form the future gastric glands (figs. 4, 5, and 6). In the course of a very short time all the gland rudiments have given origin to glandular outgrowths. Peterson recognized similar outgrowths, but he thinks that they arise from certain epithelial cells which do not reach to the free surface—lumen side of the stomach. His interpretation is obviously incorrect because, on the basis of it, he is unable to account for all the glands in older specimens. Peterson failed to discover stages similar to those shown in figures 2 and 3, and these are the critical stages in establishing the number of glands for any given specimen.

7. As the glandular outgrowths invade the mesodermic tissue there is an actual rotation of their cells (fig. 6 and 7). In many outgrowths it is not possible to distinguish cell boundaries, but in instances where they are evident, it is apparent that the shifting movements of the nuclei are but a rotation of the cells that contain them (Scammon, '15). The rotation processes are confined chiefly to the cells at the lower two-thirds of the glandular outgrowth. The fundic portion of the gland takes on a flask-like form as a direct result of these rotation processes (figs. 6 and 7).

8. With the completion of rotation, the cells at the fundic portion of the gland are placed, with reference to their long axes, at right angles to the lumen of the gland (fig. 7).

9. The fully differentiated gastric gland of Squalus acanthias is an unbranched, flask-shaped structure. The differentiation of the two cell-types does not occur.

BIBLIOGRAPHY

Bensley, R. R. 1900 The oesophageal glands of Urodela. Biol. Bull., vol. 2.

1902 The cardiac glands of mammals. Am. Jour. Anat., vol. 2..

Berry, J. M. 1900 On the development of villi in the human intestine. Anat. Anz., Bd. 17.

Bischoff, Th. W. L. 1838 Über den Bau der Magenschleimhaut. Müllers Arch. f. Anat. und Phys.

Boyd, S. 1836 On the structure of the mucous membrane of the stomach. Edin. Med. and Sur. Jour., vol. 46.

Brand, E. 1877 Beiträge zur Entwicklung der Magen- und Darmwand. Diss. Würzburg, 1877.

Edinger, L. 1877 Über die Schleimhaut des Fischdarmes, nebst Bemerkungen zur Phylogenese der Drüsen des Darmrohres. Arch. f. mikr. Anat., Bd. 13

Griffini, L., und Vassale, G. 1888 Über die Reproduktion der Magenschleimhaut. Beiträge zur pathol. Anat. u. zur allg. Pathol., Bd. 3.

Hopkins, G. S. 1895 On the enteron of American ganoids. Jour. Morph., vol. 11

Johnson, F. P. 1910 The development of the mucous membrane of the oesophagus, stomach and small intestine in the human embryo. Amer. Jour. Anat., vol. 10.

Kirk, E. G. 1910 On the histogenesis of gastric glands. Am. Jour. Anat., vol. 10.

Leydig, F. 1852 Beiträge zur mikroskopischen Anatomie und Entwicklung der Rochen und Haien. Leipzig.

Minot, C. S. 1892 Text-book of human embryology. New York.

Oppel, A. 1896 Vergleichende mikrosk. Anatomie der Wirbeltiere, Erster Teil, Jena, 1896.

Peterson, H. 1909 Beiträge zur Kenntnis des Baues und der Entwickelung des Selacherdarmes. Jena. Zeitschr. f. Naturwiss., Bd. 44.

Ross, M. J. 1902 The origin and development of the gastric glands of Desmognathus, Amblystoma and pig. Biol. Bull., vol. 4.

Scammon, R. E. 1911 Normal-plates of the development of Squalus acanthias. Heft 12, Normentaf. d. Ent. d. Wirbeltiere, Jena.

1915 The histogenesis of the Selachian liver. Am. Jour. Anat., vol. 17.

Sullivan, M. X. 1907 The physiology of the digestive tract of elasmobranchs. Bull. U. S. Bureau Fish., vol. 27.

Toldt, C. 1881 Die Entwicklung und Ausbildung der Drüsen des Magens. Sitzungsber. d. k. k. Akad. d. Wissensch., math. naturw. Kl. Abt. 3, Jahrg. 1880. Wien.

PLATES

PLATE 1

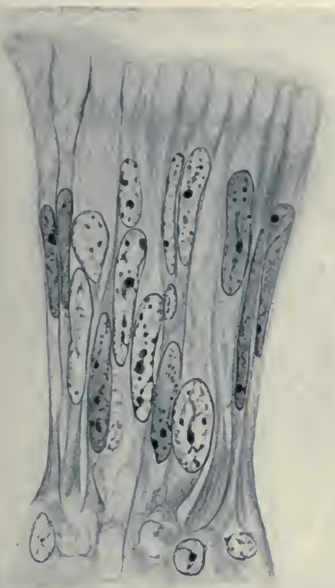
EXPLANATION OF FIGURES

The figures are all taken from the gastric epithelium of embryos fixed in a mixture of 10 per cent formalin and 2 per cent chromic acid.

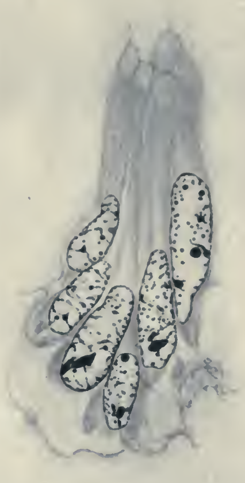
2 The earliest recognizable stage in gland development of an embryo 133 mm. long. Iron haematoxylin-orange G stain. × 1000.

3 The formation of definite gland rudiments in the epithelium of a specimen 137 mm. in length. Iron haematoxylin-orange G stain. × 1000.

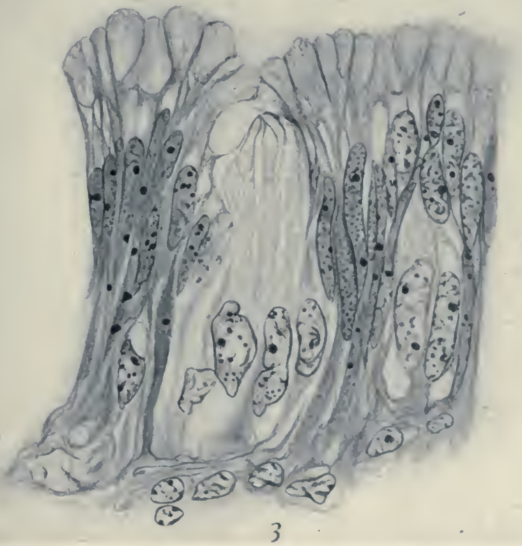
4 Outgrowth of a gland rudiment and extension into the underlying mesodermic tissue in an embryo 137 mm. long. Iron haematoxylin-orange G stain. × 1000.



2



4



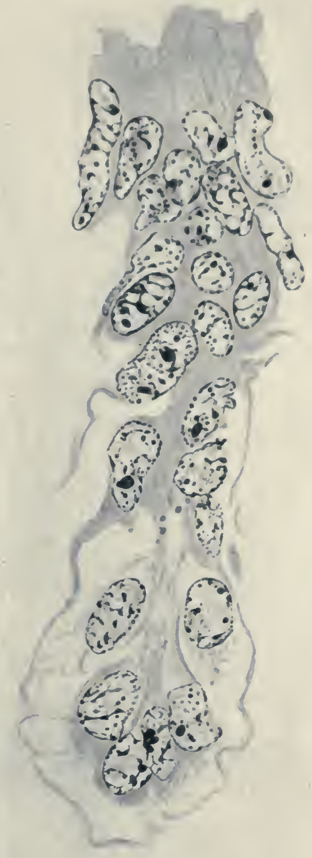
3

PLATE 2

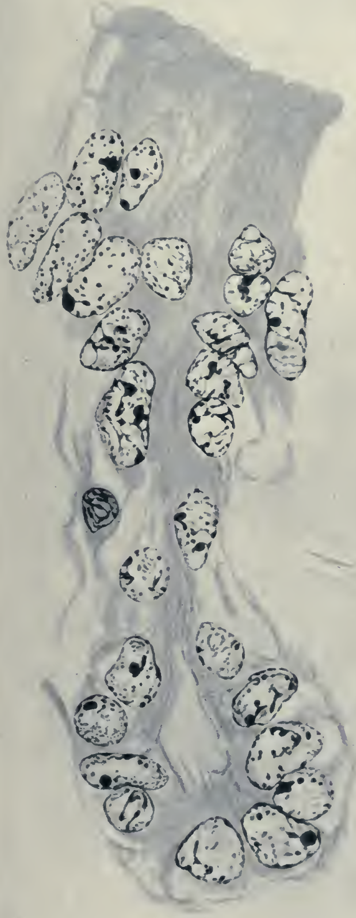
EXPLANATION OF FIGURES

5 The glandular outgrowth has assumed a tubular form in a specimen 137 mm. in length. Iron haematoxylin-orange G. × 900.

6 Shows the change in form of a glandular outgrowth as based on a rotation of cells in an embryo 137 mm. in length. Iron haematoxylin-orange G. × 900.



5

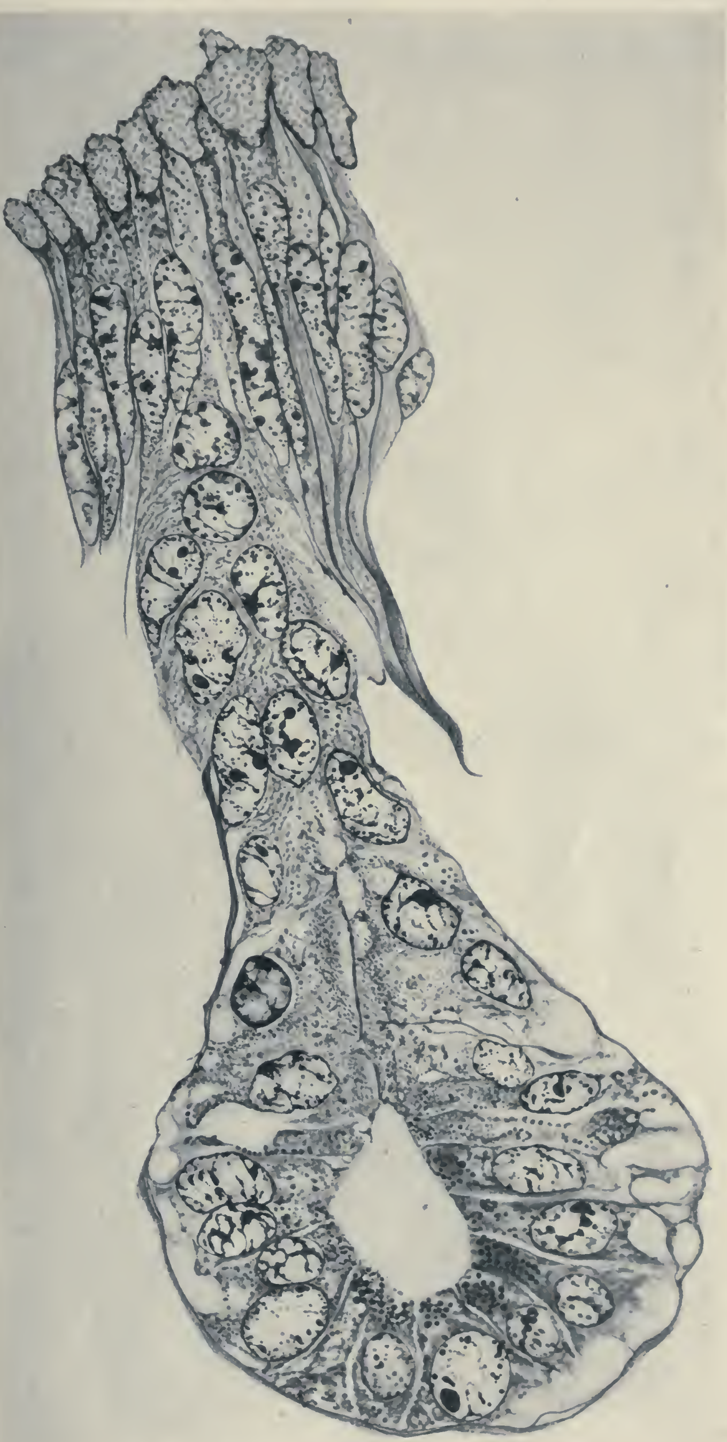


6

PLATE 3

EXPLANATION OF FIGURE

7 The fully differentiated gastric gland of a specimen 146 mm. long. Iron haematoxylin-erythrosin. × 1170.



6

No. 15

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JANUARY 6

Reprinted from THE ANATOMICAL RECORD,
Vol. 15, No. 6, January, 1919

ON THE DEVELOPMENT AND FINER STRUCTURE OF THE CORPUS ADIPOSUM BUCCAE[1]

RICHARD E. SCAMMON
Institute of Anatomy, University of Minnesota

NINE FIGURES

The corpus adiposum buccae or sucking pad is a specialized and sharply circumscribed mass of adipose tissue which lies in the cheek partially wedged between the masseter and buccinator muscles and covered externally by the superficial fascia of the face and the zygomatic muscle. Posteriorly, it is connected by a stalk with a much larger fat mass, termed by Forster ('04) the corpus adiposum malae, which is located between the temporal and the pterygoid muscles and which possesses a superficial process extending over the outer surface of the temporal muscle beneath the temporal fascia.[2]

The sucking pad was apparently first mentioned by Heister in 1732, who, thinking it was glandular in character, termed it the glandula molares. Winslow, about twenty years later, again described the structure as a gland and wrote of a series of small ducts which passed from it through the buccinator muscle to open into the oral cavity near the last molar tooth. Bichat recognized the true fatty nature of the sucking pad and referred to it in his Anatomie Générale in 1801. He is sometimes cited as the discoverer of the body. Bichat's remarks on the sucking pad are very brief and are purely incidental to a discussion on the presence of adipose tissue in early life. It is quite likely that the true nature of the body was known to anatomists before this time, although the examination of a large amount of the literature of the eighteenth century dealing with the anatomy of the fetus and child has failed to reveal any descriptions beyond those already mentioned.

---

[1] This study was carried out with the aid of a grant from the Research Fund of the University of Minnesota.

[2] The body has received many names. Besides the term applied to it by Heister, under a misconception of its nature, the structure has also been called the boule graisseuse, boule de Bichat, Wangenfettpfropf, Wangenfettpolster, Saugpolster, sucking pad, and sucking cushion. It is not clear that the B. N. A. term, corpus adiposum buccae, which I have employed here, was originally intended for this particular fat mass; in fact, it is more probable that this expression was meant to indicate the entire mass of which the corpus adiposum malae forms the main body. However, most modern authors have used the B. N. A. term in the narrow sense of the sucking pad proper, and to avoid further synonymity I have followed their example. Berg ('11), in his classification of the fat masses of the body, places the corpus adiposum buccae in the category of intermuscular fat masses together with the adipose tissue between the layers of the temporal fascia and the orbital fat.

The body as seen in the adult was figured by Burns in 1821, but it is not clear from this author's description that he regarded it as a normal structure. In 1852, Gehewe, in a Latin thesis, gave an excellent account of its gross anatomy and described its development in so far as it could be seen with the naked eye. Since this time the gross form and relations of the body have been figured and described by several authors, the most complete accounts being those of Ranke ('84), Lafite-Dupont ('00), Forster ('04), and Shattock ('09).

The phylogeny of the sucking pad has been studied in detail by Forster ('04). He finds that the entire mass of the corpus adiposum malae of the higher Primates is derived from the extra-orbital fat pad of the lemurs, which, in turn, is formed from an outgrowth of the periorbital fat mass of lower mammals. The corpus adiposum buccae, or facial extension of the corpus adiposum malae, is developed in the Primates as the orbital gland disappears and the muscles of mastication undergo partial regression.

Lehndorff ('07) investigated the chemical composition of the sucking pad and found it richer in the fats of high melting point (palmitic and stearic acids) and poorer in oleic acid than the general superficial fat. Shattock ('09), however, is of the opinion that the difference between the two is too slight to be of any great significance.

The function of the sucking pad has been discussed at length by Ranke ('84), Forster ('04), Lehndorff ('07), and Eisler ('12).

Very little has been written on the development and finer structure of the sucking pad. Gehewe ('52) found the first traces of the body in fetuses of the third month and noticed its gradual increase in size up to the time of birth. His studies were made entirely by macroscopic methods. Robin and Gimbert ('64) described the structure as appearing about the sixtieth day of fetal life as a number of clusters of small fat-cells. They found that the later growth of the body took place by the formation of new clusters as well as by the increase in the size of the earlier ones. Lafite-Dupont ('00) described the body in a fetus 12 cm. in length as consisting of a dense mass of mucous connective tissue, the fibers of which were arranged in the vertical plane of the face. This mass contained a few clusters of leucocytes. In a fetus of five months this body was transformed into a mass of adipose tissue and the embryonic mucous connective tissue had entirely disappeared. This transformation began in the central and lower part of the organ. Ranke ('84) figured and described the finer structure of the sucking pad in the late fetus and the new-born. He found it to consist of numerous lobules of unilocular fat-cells separated by broad septa of connective tissue. The whole body was surrounded by a definite capsule of fibrous connective tissue as well. A large number of blood-vessels ramified upon the outer surface of this capsule and their branches penetrated it to break up into terminal plexuses around the fat cells of the lobules. This description was confirmed by Shattock ('09), who also noted that the sucking pad was present in the fourth month of fetal life. Berg ('11) mentions that the body is in a fetus 10 cm. in length, although no fat-cells were observed at this stage.

DEVELOPMENT

The time of formation of the sucking pad, like that of most of the fat masses of the body, is subject to some variation, but the region which it will occupy later is clearly marked out in fetuses 4 or 5 cm. in total (crown-heel) length. At this time the lateral walls of the buccal cavity, which hitherto have been somewhat compressed from side to side, commence to thicken considerably with the lateral extension of the developing maxillae, so that a broad band of tissue intervenes between the epithelium lining the oral cavity and the skin covering the cheek. The margins of this mass are already occupied by sheets of developing muscle—by the anlage of the buccinator medially, and by the facial portion of the sphincter colli laterally. These muscular sheets thus form the side walls of a region which is quadrilateral in frontal section and which is bounded by the maxilla above and by the mandible and the masseter muscle below. This region is closed anteriorly by the approach of the anterior part of the buccinator muscle and the oral portion of the sphincter colli, but posteriorly it becomes continuous with the pterygoid region and through it with the orbit which is as yet incompletely enclosed by its bony walls.

The region thus outlined may be termed for convenience the buccal space. It is filled with a delicate mesenchyma which is looser meshed than that of the face generally. In this mesenchyma are embedded the parotid duct and a coarse plexus of veins. The duct passes through the facial portion of the sphincter colli, crosses transversely through the buccal space, and, after piercing the buccinator muscle, opens into the oral cavity. The venous plexus arises from the large veins at the base of the orbit and passes obliquely downward through the space. It drains in part into the facial and in part into the internal maxillary vein. The radicles of this plexus, which are of extremely irregular caliber, are surrounded by a mass of loose-meshed mesenchyma, which, however, has not differentiated sufficiently as yet to be termed preadipose tissue. A frontal section of the cheek of a fetus of this stage is shown in figure 1.

A definitive anlage of the sucking pad is generally found in fetuses from 6 to 8 cm. in total length, although sometimes it does not appear until a little later. By this time the buccal space has become somewhat narrowed through the growth of the muscles of mastication, and the individual muscles which are formed from the facial portion of the sphincter colli are clearly differentiated. The parotid duct pursues the same course through the space as it does in younger fetuses, but a definite connective-tissue sheath is now beginning to form around it. The molar glands are clearly differentiated, but have not pierced the buccinator muscle. In two specimens of this stage which I have examined the orbital inclusion was located just lateral to the buccinator muscle and anterior to the internal pterygoid (fig. 2, *O.i.*).

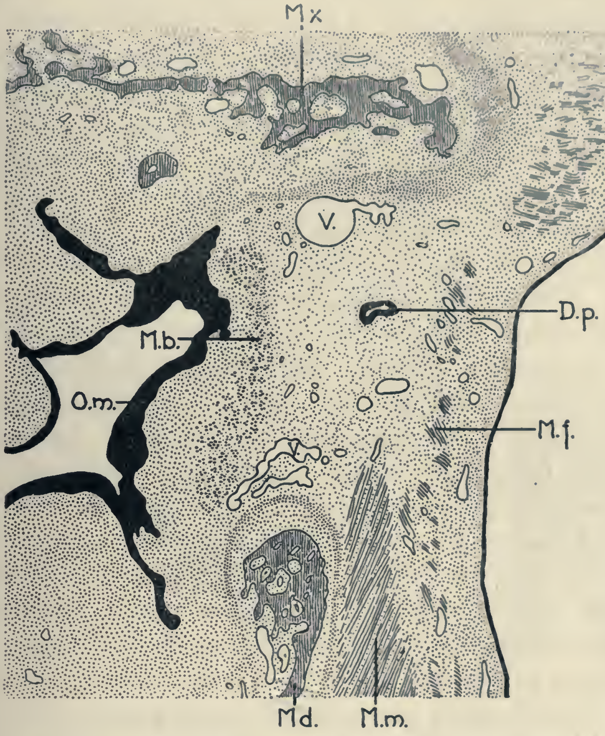


Fig. 1 Frontal section of the face of a human fetus, 60 mm. in total length, showing the region of the future sucking pad. Epithelial structures are represented in solid black, mesenchyma in stipple, muscle by short parallel lines or by coarse stipple, bone by close vertical ruling, and blood-vessels in solid outline. *D.p.*, parotid duct; *M.b.*, anlage of buccinator muscle; *Md.*, mandible; *M.f.*, anlagen of facial muscles; *M.m.*, anlage of masseter muscle; *Mx.*, maxilla; *O.m.*, oral epithelium; *V.*, venous plexus.

By this time the arrangement of the veins in this region is considerably modified. The upper part of the plexus is differentiated into several trunks which connect with the inferior veins of the orbit above, while the lower part forms vessels which drain into the facial vein below. These lower trunks represent the vena ophthalmofacialis of Gurwitsch and Sesemann or the vena facialis profunda of French authors. The middle part of the original plexus connects posteriorly with the pterygoid plexus. It is broken up anteriorly into a number of small venules which anastomose freely. The sucking pad is in the process of formation around these venules. The periphery of the mass is slightly differentiated into a capsule which is indicated more by the direction of the fibers forming it than by a condensation of the tissue. Within this capsule the mesenchyma is wide meshed and delicate except immediately around the venules, where it is somewhat condensed, forming thin sheaths about the vessels. Mixed with the preadipose tissue are a considerable number of young blood-cells. These may be the result of an accidental extravasation from the smaller vessels into the tissue of the sucking pad, but I have observed them in three of the four specimens of this stage which I have examined, and apparently they were also seen by Lafite-Dupont in a somewhat older specimen. None of the epithelial structures which penetrate the buccal space lie in the immediate region of the anlage of the sucking pad at this time. Figure 2 is a drawing of a transverse section of the left cheek and neighboring structures of a fetus 7 cm. in total length and illustrates most of the important features of the sucking pad at this stage.

In fetuses from 12 to 16 cm. in total length the corpus adiposum buccae has approached its final form and young fat lobules are commencing to replace the preadipose tissue seen in earlier stages. The mass now fills the outer part of the buccal space. It is surrounded by a definite capsule of developing fibrous tissue. Within this capsule the organ consists of a meshwork of fibers of young connective tissue in which are embedded a few developing fat lobules and the plexus of veins already described. The fat lobules are confined almost entirely to the periphery of the anterior part of the organ. They consist of preadipose tissue and true fat cells. The latter, which are quite small, are found mainly in the centers of the lobules. The duct of the parotid gland comes in contact with the capsule of the sucking pad, but does not penetrate it. The molar glands are now embedded in the substance of the buccinator muscle, but they do not come in direct contact with the capsule of the sucking pad. Small fat lobules are in the process of formation, both external to the facial musculature and fascia and also in the portion of the buccal space which is not occupied by the sucking pad and by epithelial and vascular structures. The

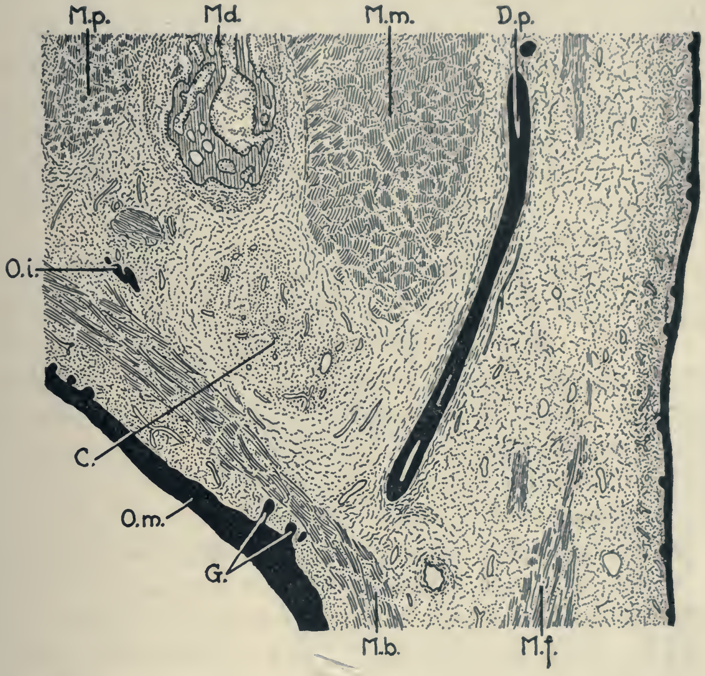


Fig. 2 Transverse section of the left cheek of a human fetus 7 cm. in total length. Method of drawing similar to that employed in figure 1. *C.*, corpus adiposum buccae; *D.p.*, parotid duct; *G.*, anlagen of molar glands; *M.b.*, buccinator muscle; *Md.*, ramus of mandible; *H.f.*, facial musculature and fascia; *M.m.*, masseter muscle; *M.p.*, internal pterygoid muscle; *O.i.*, orbital inclusion; *O.m.*, oral mucous membrane.

condition of the sucking pad at this stage is illustrated by figure 3, a frontal section passing through the extreme anterior part of the body in a fetus 15 cm. in total length.

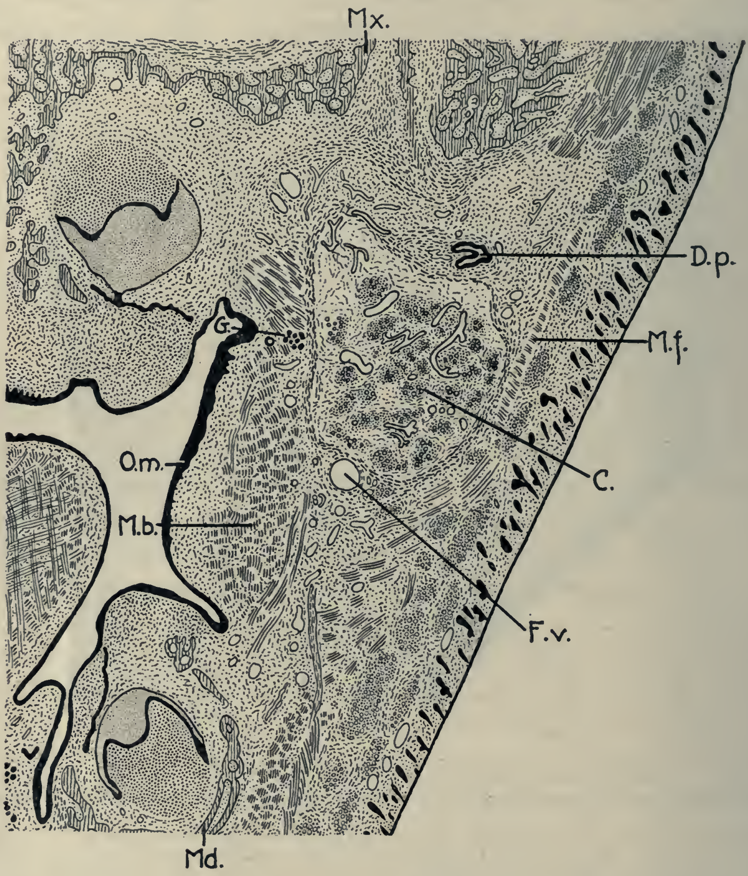


Fig. 3 Frontal section of the left cheek of a human fetus 15 cm. in total length. The section passes through the extreme anterior part of the sucking pad. *C.*, corpus adiposum buccae; *D.p.*, parotid duct; *F.v.*, facial vein; *G.*, molar glands; *M.b.*, buccinator muscle; *Md.*, mandible; *M.f.*, facial muscles and fascia; *Mx.*, maxilla; *O.m.*, oral mucous membrane.

After the sucking pad has reached the stage just described, it grows rather rapidly. It expands outward and also backward over the superficial surface of the masseter muscle and contributes considerably to the rounded form of the cheek which is so noticeable in human fetuses of the latter half of intra-uterine life. In this expansion the capsule of the body is carried outward towards the facial muscles and fascia, and the broad band of mesenchymal tissue which formerly separated these structures is reduced to a narrow sheet which contains a rich plexus of veins and a few small fat lobules. The medial portion of the capsule is also pressed inward towards the buccinator muscle, but an intermediate strip of mesenchymal tissue, which contains the bodies of the molar glands, still persists in this position. As in earlier stages, the parotid duct and the molar glands lie entirely outside the capsule of the sucking pad. They now possess definite mesenchymal envelopes which are independent of it. These relations are shown in figure 4, a drawing of a transverse section of the right cheek of a fetus 17.5 cm. in total length.

The finer structure of the sucking pad during this period of rapid growth is somewhat variable. As was pointed out in the description of the preceding stage, the formation of fat lobules takes place first at the periphery of the body and particularly in its anterior part; from this region the process extends inward and backward first into the center of the body and then into the stalk which connects it with the corpus adiposum malae. The lobules are always formed around the first branches of the venous plexus. Figure 4 shows a stage at which the peripheral lobulation of the body is well under way, while the central portion of the mass contains almost no differentiated adipose tissue. Thus the early expansion of the sucking pad is not dependent upon the formation of fat lobules, but upon the growth of the mesenchymal meshwork in which they will appear later. As the lobules are developed the connective tissue between them is reduced to the form of broad septa. It seems probable that the formation of new lobules is completed, in the majority of cases at least, by the end of the fifth fetal month. I estimate that the body contains from 250 to 350 lobules at this time.

The blood supply of the sucking pad can best be studied at this period while the lobules are still separated by broad connective-tissue septa. The arterioles which supply the body enter

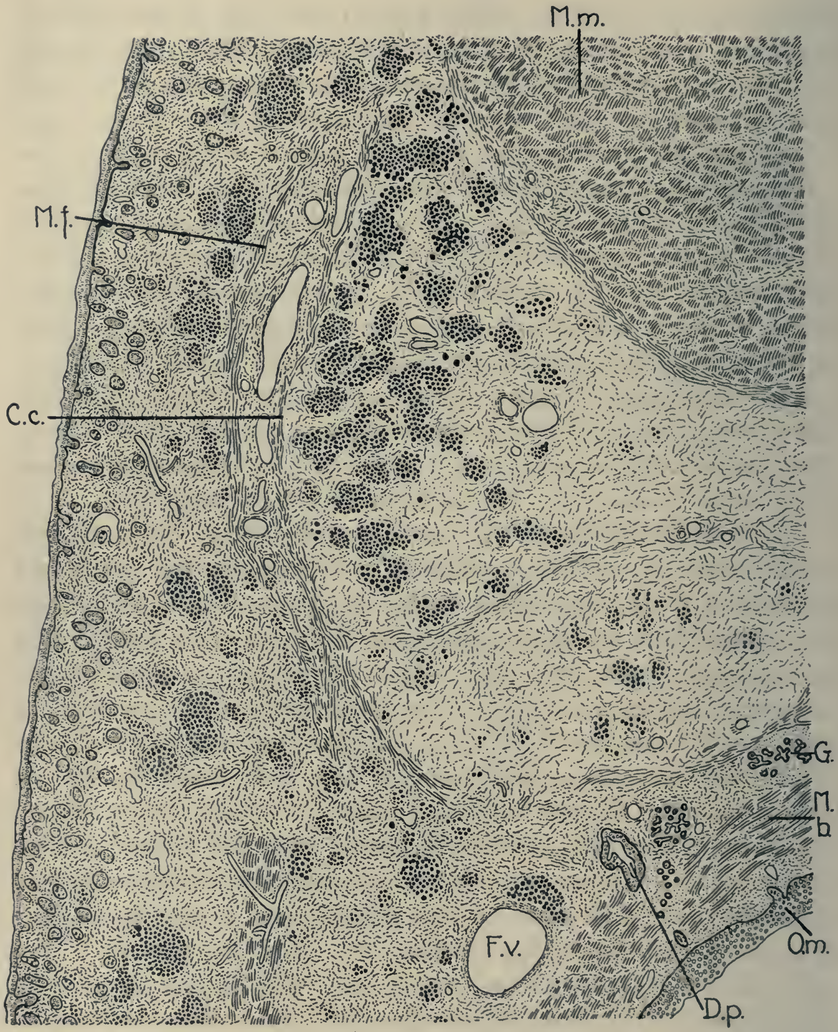


Fig. 4 Frontal section of the right cheek of a human fetus 17.5 cm. in total length. The specimen was stained with scarlet red and the colored fat droplets are represented in solid black in the drawing. *C.c.*, capsule of corpus adiposum buccae; *D.p.*, parotid duct; *F.v.*, facial vein; *G.*, molar glands; *M.b.*, buccinator muscle; *M.f.*, facial muscles and fascia; *M.m.*, masseter muscle; *O.m.*, oral mucous membrane.

its capsule from all directions, and end, after passing along the septa, by breaking up into capillary plexuses among the fat-cells of the lobules. The veins of the body are much more conspicuous than the arteries. They arise in the lobules and pass into the septa where they unite and finally form vessels of the third or fourth order. These vessels pass through the capsule and drain into the larger veins in the surrounding areolar tissue. Eventually most of the blood from the sucking pad is drained into the ophthalmofacial and facial veins. The vessels of the sucking pad are shown in figure 5—a frontal section of the body of an injected fetus 187 mm. in total length.

The subsequent changes in the body to the time of birth consist mainly in the expansion of the individual fat lobules and the reduction in thickness of the septa which separate them. With these changes the blood-vessels become much less prominent. The chronology of these later changes is subject to considerable variation, being apparently more dependent upon the nourishment of the fetus than upon its age. In some instances the fat lobules expand rapidly at an early period, so that at six months they are closely pressed against one another and are irregularly hexagonal or pentagonal in outline when seen in section. The connective-tissue septa in these cases are reduced to slender strands composed of flattened cells and fibers. In other cases this process may not take place until much later—sometimes not before the last month of fetal life. It is possible that the difficulty in suckling experienced by some premature and ill-developed infants may be due in part to the incomplete development of the sucking pad.

As has been stated, the formation of new fat lobules in the sucking pad generally ceases by the end of the fifth fetal month and the later growth of the body is due to the increase in the size of the lobules. At first this increase is brought about in part by the formation of new fat-cells at the periphery of the lobules and in part by the enlargement of the fat droplets already present. The formation of new fat-cells generally ceases in the seventh fetal month, and thereafter, as a rule, growth takes place only by the enlargement of the fat-cells which are already formed.

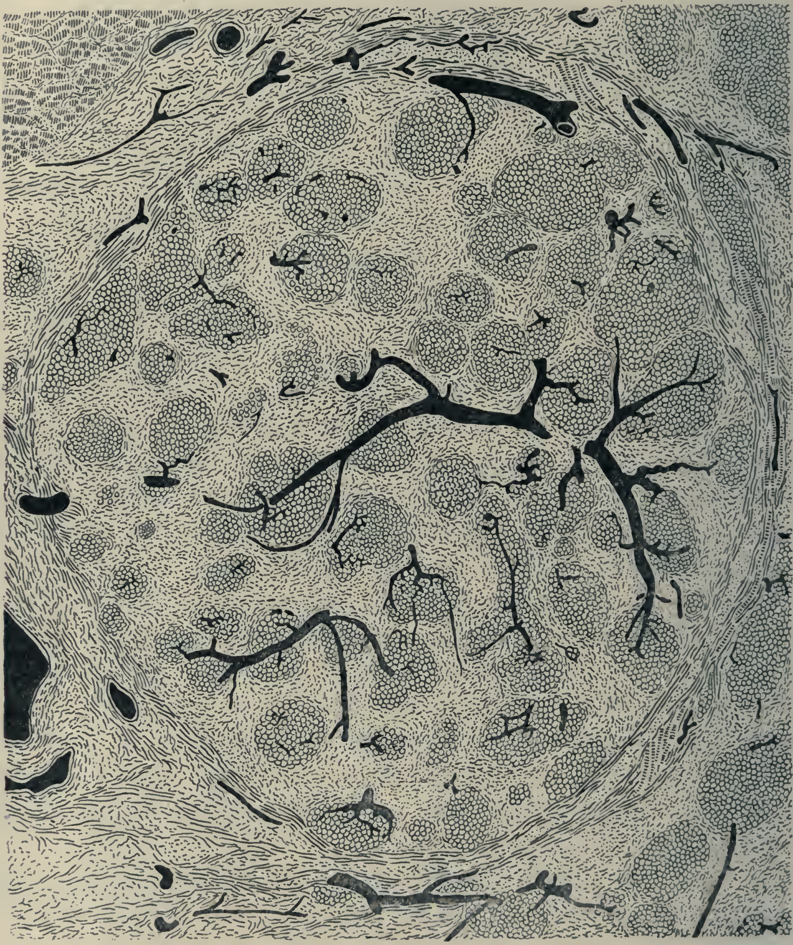


Fig. 5 A frontal section of the sucking pad of a human fetus 18.7 cm. in total length. The veins of the specimen have been injected and are represented in solid black in the drawing. The fat-cells are represented by small circles.

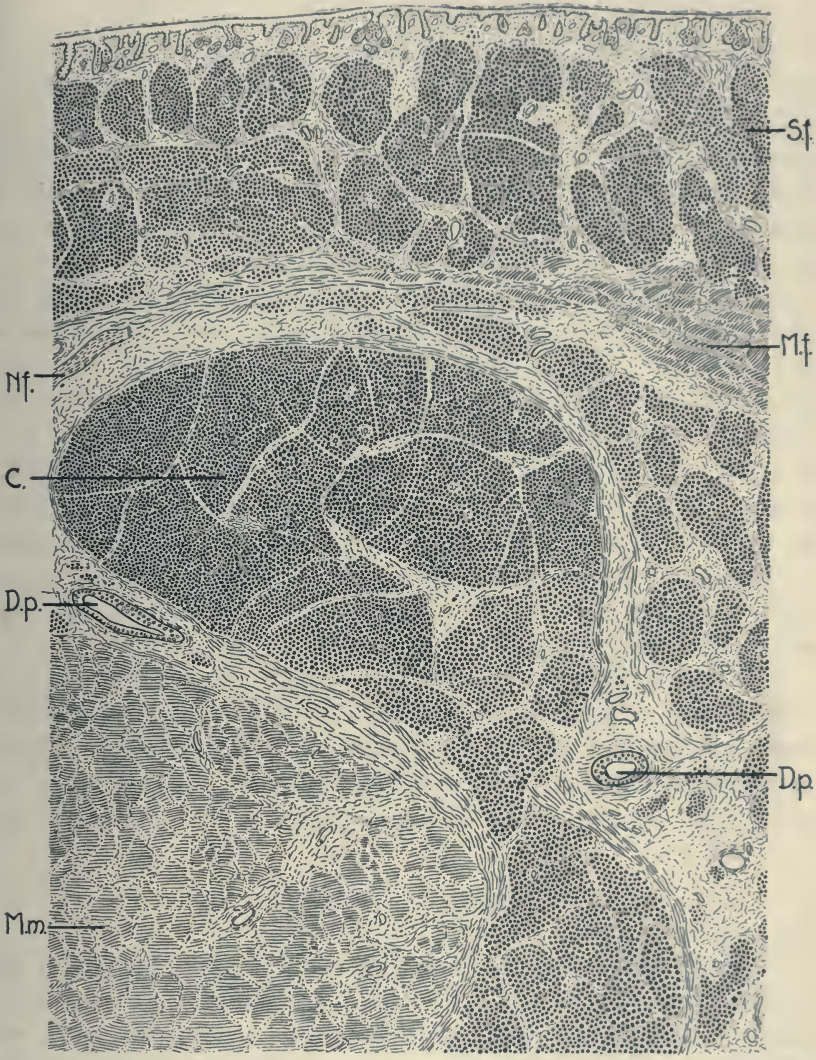


Fig. 6 A portion of a transverse section of the left cheek of a human fetus 32 cm. in total length. The section was stained with scarlet red and the colored fat droplets are represented in solid black in the drawing. *C.*, corpus adiposum buccae; *D.p.*, parotid duct; *M.f.*, facial musculatur and fascia (the leader enters the zygomatic muscle); *M.m.*, masseter muscle; *N.f.*, branch of facial nerve; *S.f.*, superficial fat lobules.

STRUCTURE OF THE SUCKING PAD AT BIRTH

At birth the sucking pad is a prominent structure of the cheek. In well-nourished individuals it is expanded to such a degree that it pushes the buccinator muscle inward towards the oral cavity and forms a prominent elevation laterally on the external surface of the face. The expansion of the body has forced its capsule outward against the superficial fascia of the face and inward against the fascia covering the buccinator muscle. Only a small cleft containing areolar tissue and blood-vessels separates the capsule of the body from these fascial planes. It is due to the presence of this space that the sucking pad is so easily dissected out in the new-born. This separation between the capsule and the investing fascia is so readily accomplished that the body was at one time described as partially surrounded by a bursa (Verneul, '57). Sections of this region, however, show no evidence of such a structure. Figure 7 is of a frontal section of the face of a very well-developed and nourished new-born child weighing 4050 grams. It shows the sucking pad in a high state of development.

The finer structure of the body at birth is almost identical with that of the general superficial fat except that the interlobular septa are perhaps a little narrower and are somewhat radially arranged in regard to the center of the body, while those of the superficial fat are placed at right angles to the surface of the skin (fig. 8).

POSTNATAL HISTORY

Comparatively little is known of the postnatal history of the corpus adiposum buccae. Gehewe ('52) stated that the body persists throughout life and that he had observed it in the emaciated cadaver of a woman over sixty years of age. Robin and Gimbert ('64), on the other hand, found little change in its size during the first four or five years of life, but concluded that after that time it diminished with age and with disease. Lafite-Dupont ('00) also thought that the body became smaller with age. Ballantyne ('91) agrees with Gehewe that the body persists in maturity, as also does Shattock ('09). The survival of the structure during the wasting diseases of infancy is a common clinical observation and has been commented upon by Ranke ('84), Lehndorff ('07), and others. As has been stated, Allen Burns ('21) was apparently the first observer to figure the structure accurately in the adult. Modern treatises on adult human anatomy usually give little or no description of the body and sometimes use the term corpus adiposum buccae for the general fat mass of the cheek and not for the sucking pad proper. However, the body is briefly described in connection with the mouth by Jonnesco in Poirier and Charpy's Traité d'Anatomie and in detail by Eisler in Bardeleben's Handbuch.

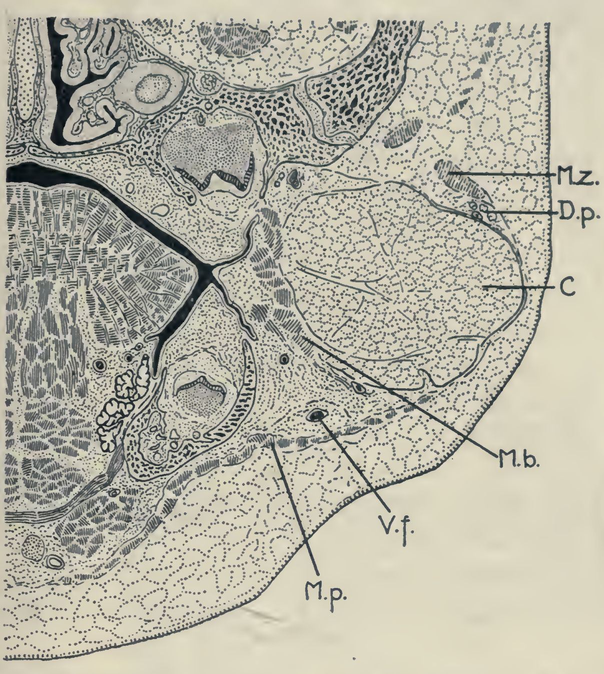


Fig. 7 Frontal section of a portion of the face of a very well-developed and nourished new-born infant weighing 4050 grams. *C.*, corpus adiposum buccae; *D.p.*, parotid duct and accessory parotid glands; *M.b.*, buccinator muscle; *M.p.*, platysma muscle and fascia; *M.z.*, zygomatic muscle; *V.f.*, facial vein. × 2½.

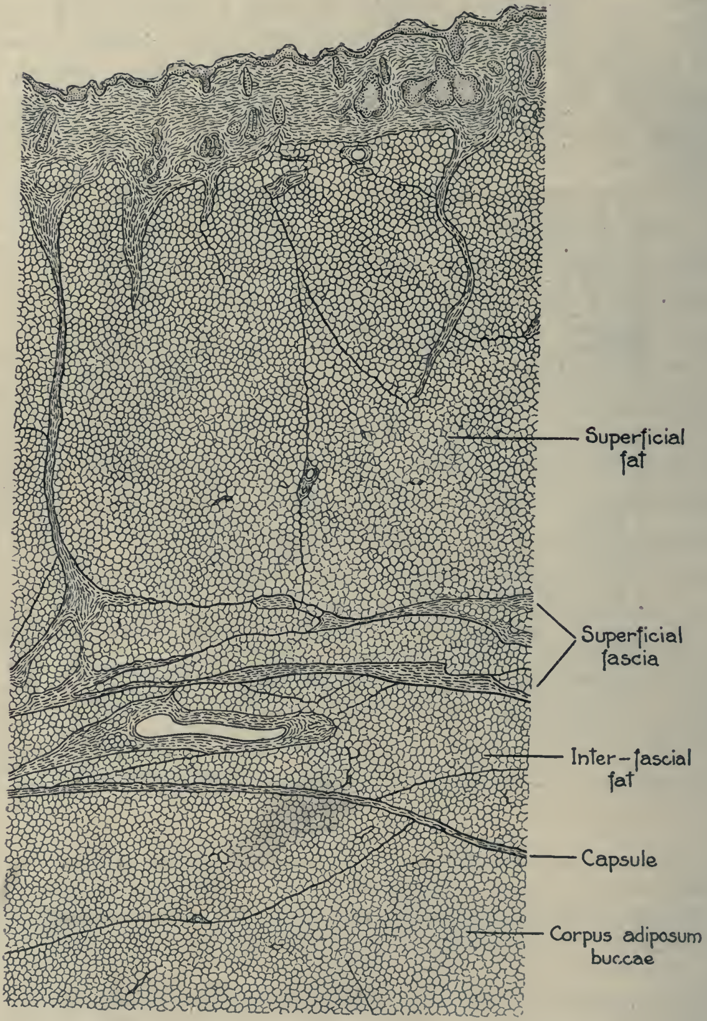


Fig. 8 A section passing through the skin, superficial fascia, and outer part of the sucking pad of a well-developed and nourished new-born infant weighing nearly 4000 grams.

In order to determine the usual condition of the sucking pad in the adult, a series of forty-two cadavers was examined in the dissecting room. The body was well developed in thirty-four of these cases and in two other instances it was present and well developed on one side of the face and practically absent on the other. This series of cases included the bodies of individuals from about twenty to about sixty years of age. So far as could be observed there was no relation between the size of the sucking pads and the age of the individual. A number of the cadavers of this series were of persons who had died in an advanced stage of tuberculosis; in some of these cases the superficial adipose tissue of the body was reduced to the minimum, but the sucking pads showed little or no reduction in size. It is evident that wasting disease, in the adult as in the suckling, has little effect upon the sucking pad.

The body in the adult may occupy the fossa bounded by the masseter, the buccinator, the zygomatic, and the platysma and risorius muscles, or it may extend forward and outward over the external surface of the masseter. The parotid duct, as in the fetus and the infant, may either pass cranial to the body or may lie in a deep groove on its superficial surface. Figure 9 shows several sketches of the body in adult cadavers. Figure 9, *C*, is of an individual who died in an advanced stage of phthisis.

Frozen sections of sucking pads of adults show practically the same structure as that seen in the infant.

PHYLOGENETIC SIGNIFICANCE

It has been suggested that the corpus adiposum buccae of the higher Primates represents the framework of the orbital gland which is so well developed in the Carnivora and of which the molar glands of man are a vestige. This view was first advanced, I think, by Lafite-Dupont ('00). In my opinion, neither the phylogenetic studies of Forster on the sucking pad nor my observations on the development of this structure support this hypothesis.

Forster finds that the sucking pad is a specialized portion of a fat mass which takes its origin from the extra-orbital fat body of the lemurs and which only secondarily enters the buccal region in the higher Primates. It is thus a body from another area which invades the region of the orbital gland and fills the space formerly occupied by that organ, but it is in no sense the remains of it.

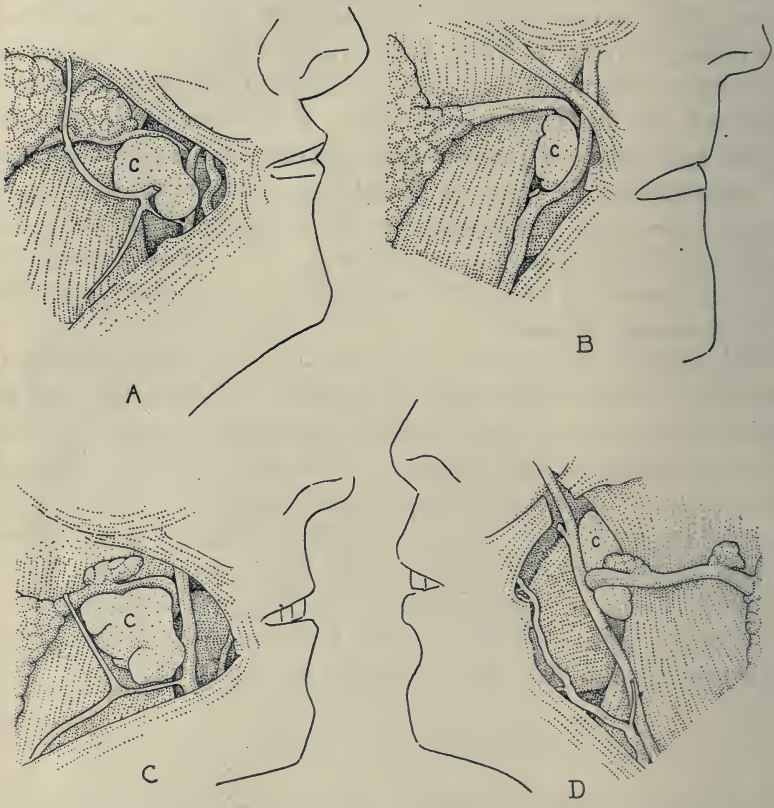


Fig. 9 Four sketches of dissections of the sucking pad in adult cadavers. *C.*, corpus adiposum buccae.

The sucking pad, in its development, is built up around a venous plexus and not around any element of the orbitoparotid gland complex. The epithelial elements of this complex in man (the parotid duct, the orbital inclusion, and the molar glands) do enter the area which I have termed the buccal space, but they do not enter the territory which is later to be incorporated in the sucking pad and they do not pierce the capsule of this structure after it is differentiated. In fact, the molar glands, which are considered to be the vestiges of the orbital gland, do not enter the buccal space at all until long after the sucking pad has been differentiated and a definite capsule has formed around it.

In Carmalt's paper ('13) on the anatomy of the adult salivary glands in man the statement is made that "The molar glands, when present, are for the most part embedded in the ento-masseteric fat mass of the 'sucking pad.'" While these glands lie in the loose adipose and areolar tissue of this region which fills the space between the corpus adiposum buccae and the muscles on either side of it, I have not observed them penetrating the capsule of the sucking pad proper, and the relation between them and the sucking pad is one of juxtaposition only. I think, therefore, that it may be safely concluded that while the sucking pad replaces the orbital gland in position in the higher Primates and in man, it is not to be regarded as a vestige of that structure.

SUMMARY

1. The corpus adiposum buccae is a sharply circumscribed mass of fat lobules which are formed around the radicles of the middle part of the venous plexus which connects the orbital veins with the superficial veins of the face. It is differentiated within a fairly well-marked area of the cheek which may be termed the buccal space.

2. The general region in which the sucking pad arises is mapped out in fetuses 4 or 5 cm. in total length and a definitely encapsulated area is well marked in fetuses 8 to 10 cm. in length. Fat-cells appear at this stage or a little later. They are arranged in lobules which are first found in the periphery of the anterior part of the body.

3. The body grows rapidly after the encapsulated area has been established. Most of this early growth is due to the expansion of the enclosed mesenchymal and preadipose tissue and not to the growth of fat-cells.

4. The later growth of the body is due to an increase in its fat content. This is brought about: *a*) by the increase in the number of fat lobules; *b*) by the formation of new fat-cells, and *c*) by the growth of the individual fat-cells. The formation of fat lobules generally ceases by the end of the fifth fetal month. The formation of new fat-cells ceases at a variable time in later fetal life, generally in the sixth or seventh fetal month, but sometimes not before the last fetal month.

5. The finer structure of the fully developed corpus adiposum buccae does not differ from that of ordinary superficial adipose tissue except that the interlobular septa are somewhat narrower and are arranged radially in the body.

6. The body persists in adult life in the large majority of cases. The presence of the sucking pad in later life is apparently not dependent on nutrition, as it may be found well developed on one side of the face and absent on the other in the same individual. It is also found well developed in individuals dead of wasting disease.

7. Observations on the development of the sucking pad in man offer no support to the theory that the body represents the remains of the orbital salivary gland. The sucking pad is developed quite independently of the parotid duct, the molar glands, and the orbital inclusion, and these structures never penetrate it. The molar glands do not approach the area in which the sucking pad is formed until some time after that structure is well established.

BIBLIOGRAPHY

Ballantyne, A. N. 1891 An introduction to the diseases of infancy. Edinburg.

Berg, W. 1911 Über die Anlage und Entwickelung des Fettgewebes beim Menschen. Zeitschr. f. Morphol. u. Anthropol., Bd. 13.

Bichat, F. M. X. 1801 Anatomie générale, appliquée a la physiologie et a la médecine. Paris.

Burns, A. 1821 Observations on the chirurgical anatomy of the head and neck. Edinburg.

Carmalt, C. 1913 A contribution to the anatomy of the human adult salivary glands. Studies in cancer and allied subjects conducted under the George Crocker special research fund at Columbia University, vol. 4.

Eisler, P. 1912 Die Muskeln des Stammes. (Bd. 1, 2. Abt., 1. T., Handb. d. Anat. d. Menschen, K. v. Bardeleben.)

Forster, A. 1904 Über die morphologische Bedeutung des Wangenfettpfropfes. Arch. f. Anat. u. Ent.

Futamura 1906 Über die Entwicklung der Facialismuskulatur des Menschen. Anat. Hefte, Bd. 30.

Gehewe, W. 1852 De corpusculo quodam adiposo in hominum genis obvio. Diss. Dorpat.

Gurwitsch, M. 1883 Über Anastomosen zwischen den Gesichts- und Orbitalvenen. Arch. f. Ophthalmol., Bd. 24.

Heister, L. 1732 Compendium anatomicum. Norimbergae.

Jonnesco, T. 1901 Tube digestif. (Poirier et Charpy. Traité d'Anatomie Humaine, T. 3, Fasc. 1, 2. Éd.)

Lafite-Dupont 1900 La gland infra-orbitaire et la boule graisseuse de Bichat. Bibl. Anat., T. 8.

Lehndorff, H. 1907 Über das Wangenfettpolster der Säuglinge. Jahrb. f. Kinderheilk., Bd. 66.

Ranke, H. 1884 Ein Saugpolster in der menschlichen Backe. Arch. f. path. Anat., Bd. 97.

Robin, C., et Gimbert 1864 De la boule graisseuse de Bichat. Compt. Rend. Soc. Biol., Paris, T. 16.

Sesemann, E. 1869 Die Orbitalvenen des Menschen und ihre Zusammenhang mit den oberflächlichen Venen des Kopfes. Arch. f. Anat. u. Physiol.

Shattock, S. G. 1909 On normal tumour-like formations of fat in man and the lower animals. Proc. Roy. Soc. Med., Rept. for Session 1908–1909, Patholog. Sect.

Verneuil 1857 Une nouvelle bourse sereuse de la face. Bull. Soc. Anat. Paris, Sér. 2, T. 2.

Winslow, J. B. 1753 Expositio anatomica structurae corporis humani. Francofurti et Lipsiae.

No. 16

SOME GRAPHS AND TABLES ILLUSTRATING THE GROWTH OF THE HUMAN STOMACH *

RICHARD E. SCAMMON, Ph.D.
Institute of Anatomy, University of Minnesota
MINNEAPOLIS

The importance of an accurate knowledge of the anatomy of the stomach in early life has long been appreciated as is evidenced by the numerous publications on the subject. In recent years, particularly, our concepts of the form and position of the infant's stomach have been entirely recast by the results of the series of roentgenographic studies initiated by Levin and Barret[1] and continued by a number of investigators in this country and elsewhere. A number of studies have also given us at least a working knowledge of the finer structure of the organ in childhood. Yet, regarding the actual growth of the stomach, there is probably less known than of any of the other larger organs of the body. Although there are a number of scattered observations on the weight of the stomach at various periods of life, apparently no attempt has ever been made to bring these together or to follow out the general scheme of the mass increase of the organ. The same remark holds true for the increase in the area of the gastric mucosa. Regarding the growth of gastric capacity, there is, of course, a very large quantity of data, and this has been summarized from time to time in the studies of Pfaundler,[2] Borie,[3] Marfan,[4] Figueira,[5] and others. It is, however, over fifteen years since a new synthesis of this material has been attempted, and a very large amount of new data on the subject has accumulated since that time. This condition seems to warrant the presentation of a series of statistics on stomach growth together with a graphic analysis of some of the more important material.

---

* This study was carried out with the aid of a grant from the Research Fund of the University of Minnesota.

[1] Levin, G., and Barret, G.: L'estomac du nourrisson. Forme-Limite inférieure—Mode de replissage et d'évacuation, Presse méd. **40**:503, 1906.

[2] Pfaundler, M.: Ueber Magencapacität und Gastrectasie im Kindesalter, Biblioth. med. Suppl. 4, Part 5, 1898.

[3] Borie, P. R.: L'estomac du nourrisson, Anatomie et Physiologie, Thèse Paris, 1899.

[4] Marfan, A. B.: Traite de l'allaiment et l'alimentation des enfants du premier age, Paris, 1903, Ed. 2, p. 293.

[5] Figueira, F.: Eléments de séméiologie infantile, Paris, 1903, p. 206 et seq.

1. GROWTH OF THE STOMACH IN WEIGHT

Somewhat over 1,400 determinations of the net weight of the stomach are available for study. Of these, 839 are weighings of adult stomachs (of individuals from 20 to 60 years of age), 476 are of individuals of the first two decades of postnatal life and the remainder are of new-born infants and fetuses. Unfortunately, only a portion of this material is available in the form of individual cases. The data on the weight of the stomach in the latter part of childhood and in adolescence come almost entirely from the tables of Boyd,[6] whose findings were published only in summary, and whose original records were completely destroyed many years ago. This makes it necessary to group the cases of this period in larger divisions of time than would otherwise be used. However, Boyd's material for early childhood is arranged in small time units which allow a detailed study of this period. The data on stomach weight are shown in tabular form in Table 1, and Figures 1 to 5, inclusive, are graphs based on them.

TABLE 1.—The Growth of the Stomach in Weight in Fetal and Postnatal Life (1,436 Cases) *

Age	Number of Cases	Average Net Weight, Gm.	Average Relative Weight	Average Weight in Terms of Weight of Stomach of New-Born	Average Weight in Terms of Weight of Adult Stomach
3d fetal month...........	2	0.125	0.250	0.019	0.0008
4th fetal month...........	2	0.325	0.330	0.050	0.002
5th fetal month...........	5	0.512	0.224	0.078	0.003
6th fetal month...........	6	0.99	0.303	0.152	0.006
7th fetal month...........	11	1.89	0.275	0.290	0.012
8th fetal month...........	13	2.77	0.301	0.426	0.017
9th fetal month...........	7	3.90	0.227	0.600	0.025
10th fetal month...........	8	4.85	0.190	0.746	0.031
Newborn.....................	167	6.49	0.225	1.00	0.042
0 to 3 months..	34	10.9	0.371	0.167	0.070
3 to 6 months.............	39	14.1	0.417	2.17	0.091
6 to 12 months.............	71	18.3	0.352	2.81	0.118
1 to 2 years...............	62	27.1	0.434	4.17	0.175
2 to 4 years...............	55	37.9	0.439	5.83	0.245
4 to 7 years...............	48	51.8	0.438	7.97	0.335
7 to 14 years...............	35	89.8	0.487	13.81	0.581
14 to 20 years...............	32	128.9	0.430	19.98	0.834
20 to 60 years...............	839	154.5	0.309	23.77	1.000

* Based on data collected by Arnovljevic, S.: Das Alter, die Grössen und Gewichtsbestimmung der Foetalorgane, Dissert. München, 1884; Boyd, R.: See Footnote 6; Brandt, E.: Das Alter, die Grössen und Gewichtsbestimmung der Foetalorgane, Dissert. München, 1886; Clendinning, J.: Facts and Inferences Relative to the Condition of the Vital Organs and Viscera in General as to Their Nutrition in Certain Chronic Diseases, Medico-Chir. Tr. **21**:32, 1836, and the author.

The first part of Table 1 and the graphs shown in Figures 1 and 2 illustrate the growth of the stomach in fetal life and its weight at birth. The youngest stomachs weighed were of two fetuses of the latter part of the third fetal month. At this time the weight of the organ is 0.125 gm. This weight is almost tripled in the fourth fetal month and is more than quadrupled in the fifth fetal month. In the sixth month the average weight of the organ is approximately 1 gm., or about eight times that in the third. In the seventh month the average weight has risen to nearly 2 gm., and in the eighth to 2.77 gm. The average weight of the organ in the ninth fetal month is approximately 3.9 gm., in the tenth month 4.8 gm., and in the full-term new-

---

[6] Boyd, R.: Tables of the Weights of the Human Body and Internal Organs in the Sane and Insane at Various Ages, Arranged from 2,640 Postmortem Examinations, Phil. Tr. Roy. Soc., London **151**: 1861.

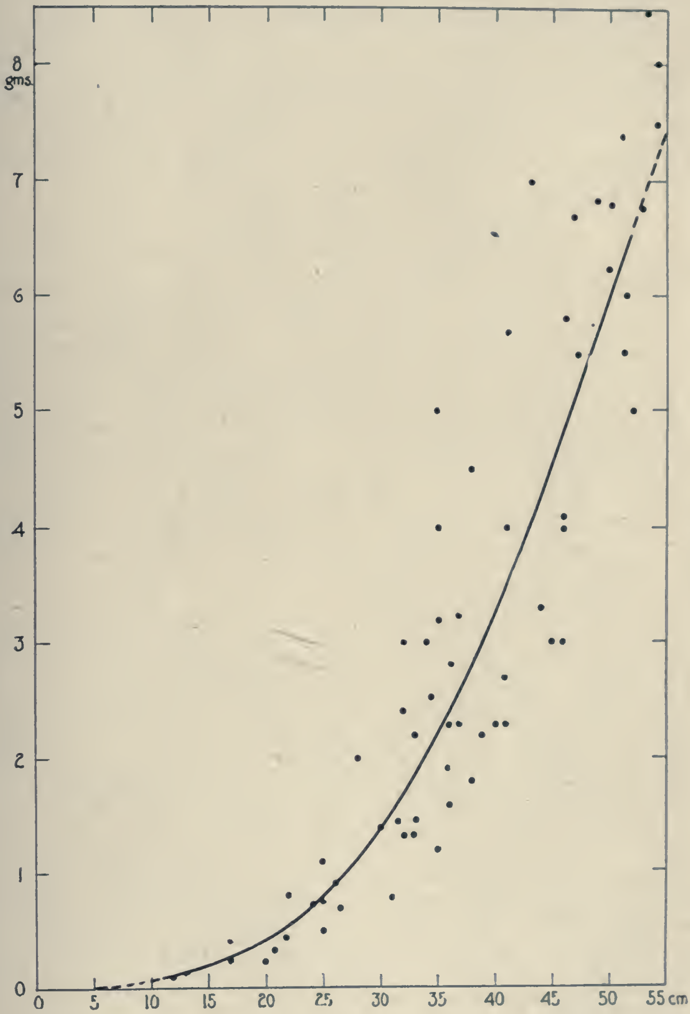


Fig. 1.—A field graph and curve illustrating the growth of the stomach in net weight during fetal life. Abscissa: total body length in centimeters; ordinate: net weight of stomach in grams. The distribution of the individual cases is shown by dots. The curve of average weight is based on the figures presented in Table 1. The position of the determining points on this curve have been weighted for the distribution of cases as to body length and the curve has been slightly smoothed by inspection.

born approximately 6.5 gm. The fetal growth of the organ is evidently a very rapid one. In the early part of prenatal life the organ approximately doubles its weight with each fetal month. Thereafter the rate of growth drops to about 40 per cent. per month for the last third of prenatal life.

Looking at the process of growth of the stomach in fetal life as a whole, we find that the organ has acquired about one-fiftieth of its natal weight in the third fetal month, nearly one-sixth in the sixth, between one-third and one-fourth in the seventh, and over two-fifths in the eighth. The exact determinations of this ratio of the last two months are difficult because of the uncertainty of classifying specimens of this period, but by the end of the ninth fetal month the organ has certainly acquired two-thirds of its birth weight.

The relative weight of the stomach, that is, the per cent. which the organ forms of the entire body weight, seems to show no consistent change in prenatal life, although we encounter considerable variations in the monthly average in this as in all other organs. The relative weight in the latter part of the third fetal month is 0.250, and in the new-born 0.225. In the tenth fetal month this falls as low as 0.190, and in the fourth month it rises to 0.330, but these variations show no constant trend either above or below the general average of about 0.250 for the entire fetal period. They are probably to be regarded as due to fluctuations in the body weight rather than to changes in the rate of the stomach growth as compared with that of the growth of the body as a whole.

In the full-term new-born the average weight of the stomach is approximately 6.5 gm., which is somewhat more than 0.2 of 1 per cent. of the total weight of the body. This weight is the average of 167 cases. The variation from this average in individual cases is very great and stomachs weighing as little as 4 gm. or as much as 8 gm. are not uncommon in apparently normal and full-term new-born children.

The growth of the stomach in postnatal life is quite different from that of most of the organs of the body. The increase of the body in weight from birth to early maturity is generally in the neighborhood of twenty fold or a little more. Most of the larger viscera, such, for example, as the liver, the heart, the kidneys and the spleen increase about twelve times in weight in the same interval. In other words, the growth rate of these viscera is but little more than one half that of the entire body in the period between birth and the early part of the third decade. The stomach, on the other hand, increases from an average weight of 6.49 gm. at birth to an average weight of 154.5 gm. in the adult, or nearly twenty-four fold. Thus its growth rate in postnatal life is about twice that of many of the larger organs and is equal to or a little greater than that of the body as a whole. So far as I am aware, only two other organs of the body approach the stomach in rate of postnatal growth. These are the parotid glands and the pancreas. But the growth of the parotid glands does not equal that of the stomach; and that of the pancreas, while approaching it, takes place at a somewhat different period in postnatal life. These peculiarities of the stomach's growth in postnatal life are illustrated in Tables 1 and 2 and by the graphs in Figures 2 and 5, inclusive. Starting with an average natal weight of about 6.5 gm., the stomach increases to an average weight of nearly 11 gm. in the first trimester, and a weight of about 14 gm. in the second trimester. The average weight in the second half of the first year is over 18 gm. In the second year the average weight of the organ is approximately 27 gm., and in the third and fourth years nearly 40 gm. Thus, the stomach more than doubles its weight in the first six months, and triples it by the end of the first year. By 2 years of age the birth weight of the organ has been increased about fivefold. By far the most rapid growth period is in the first trimester after birth.

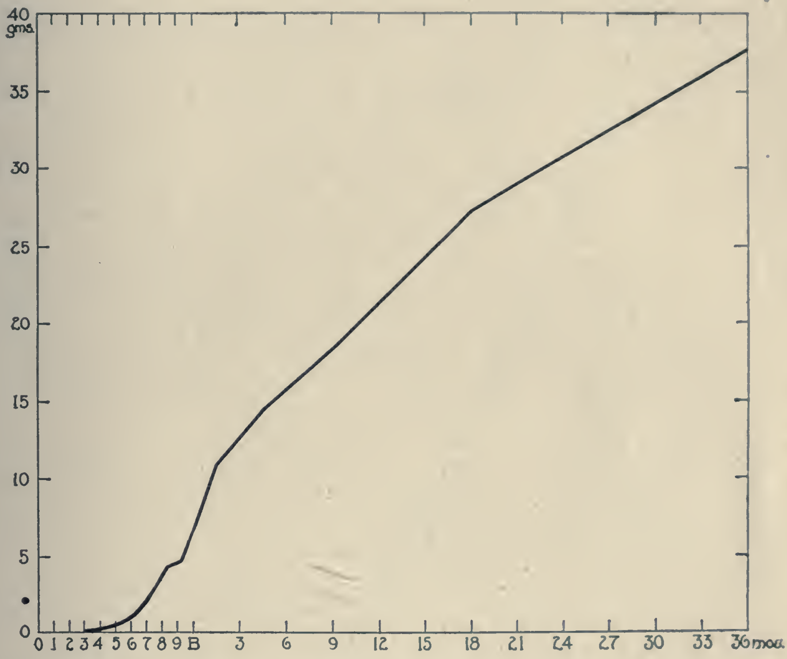


Fig. 2.—A curve illustrating the growth in the net weight of the stomach from the third fetal month to the close of the second year. Abscissa: age in months (fetal or postnatal); ordinate: weight of the stomach in grams. The point "B" on the base line indicates birth. Based on the material presented in Table 1.

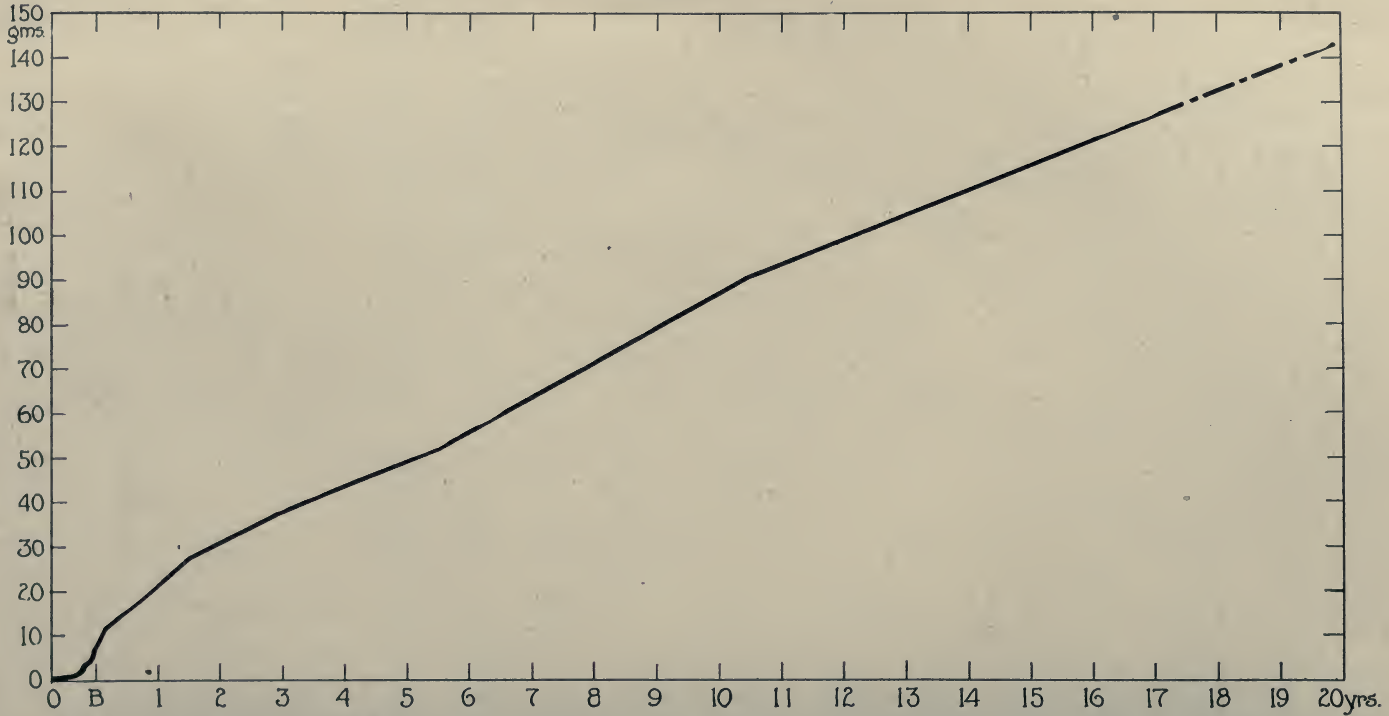


Fig. 3.—A curve illustrating the growth of the stomach in net weight from early fetal life to 20 years. Abscissa: age in years; ordinate: stomach weight in grams. B, on the base line, indicates birth. Based on the material presented in Table 1.

The growth of the stomach in net weight from infancy to maturity is illustrated by the curve shown in Figure 3. As seen in this figure, the rapid weight increment of infancy is much reduced after the first two years, although the organ continues to increase in weight throughout the first and second decades. There is apparently a slight drop in the rate of absolute growth about the close of the first period of childhood and a slight increase in the years before puberty. These changes would probably be seen in a more pronounced fashion were the material arranged in smaller time units, as similar changes are noticeable not only in most curves of the growth of the body as a whole, but also in curves of the growth in weight of many of the organs.

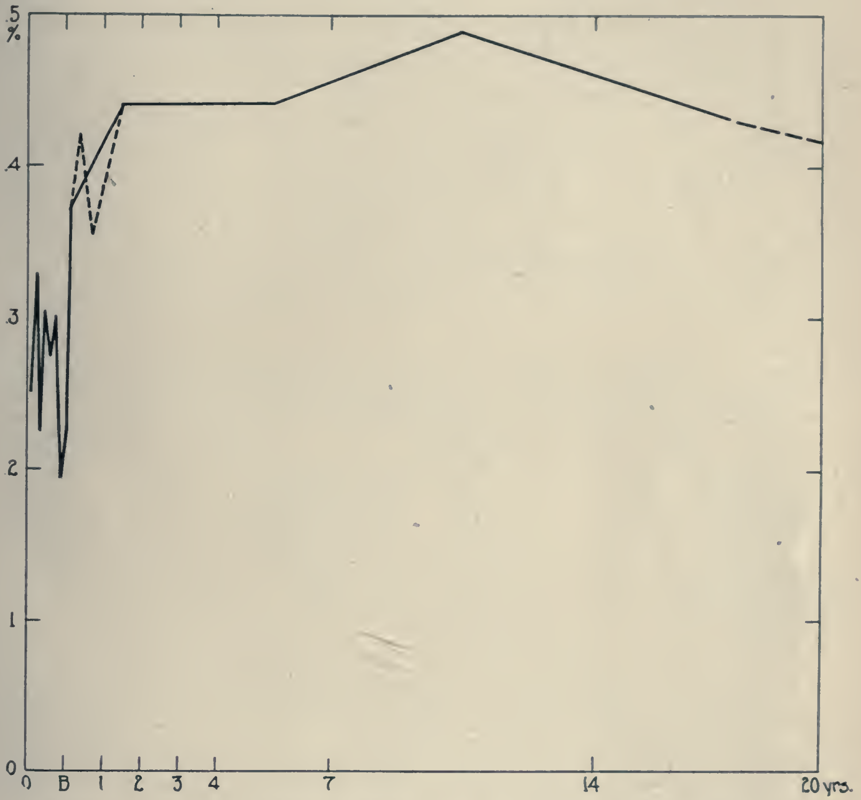


Fig. 4.—A curve illustrating the changes in the relative weight of the stomach from the third fetal month to 20 years. Abscissa: weight of the stomach in per cent. of the total body weight. Ordinate: age in years. Based on the data presented in Table 1.

The rate of percentage weight increment, that is, the percentage of its weight which the stomach gains in a given period, is shown in

Table 2. In this table the percentage increment is calculated on the basis of the month. These calculations are based on values which have been determined from the originals of the graphs shown in Figures 1, 2 and 3, and are therefore only approximations. But they are sufficiently accurate to illustrate the general changes in the rate of increment which take place in fetal and postnatal life.

The rate of increment in the first trimester is about 31 per cent. per month, being about three fourths that observed in the last three months of fetal life. This rate drops to about 9 per cent. in the second trimester and to about 5 per cent. in the latter half of the first year. This again decreases to a little over 2 per cent. in the second year and to a little over 1 per cent. in the remainder of childhood. In the second decade the average rate of increment is about 0.5 per cent. per month. So far as I am aware, the continuance of a rate of increment approaching that of the last fetal months into the first trimester of postnatal life is found in no organ of the body except the stomach.

TABLE 2.—Approximate Rate of Monthly Increment in Stomach Weight During Fetal and Postnatal Life (Calculated from Graphs)

Age	Per Cent.	Age	Per Cent.
4th fetal month	100.0	9 to 12 months	5.1
5th fetal month	100.0	12 to 18 months	4.7
6th fetal month	62.0	18 to 24 months	2.2
7th fetal month	50.0	2 to 3 years	1.9
8th fetal month	43.0	3 to 4 years	1.2
9th fetal month	40.0	4 to 5 years	1.0
10th fetal month	44.0	5 to 10 years	1.3
Birth to 3 months	31.0	10 to 15 years	0.5
3 to 6 months	8.8	15 to 20 years	0.4
6 to 9 months	5.3		

The relative weight of the stomach in postnatal life is illustrated by the figures in the fourth column of Table 1, and by the graph shown in Figure 4. The great fluctuations in the body weights of cases coming to necropsy makes this quantity somewhat variable, particularly in early childhood. In the new-born the relative weight of the organ is 0.225. This is almost doubled in the first six postnatal months, rising to 0.417. In the second half of the first year the relative weight apparently drops again to 0.352, but this decline is probably due to the nature of the material in the particular cases which were examined rather than to an actual constant change in the relation of stomach weight to body weight in all cases of this period. After the first year the relative weight advances slowly to nearly 0.5 per cent. at puberty. Thereafter a decrease sets in which eventually reduces the maximum relative weight of puberty by about one third. The relative weight of the adult stomach averages 0.309, being about one fourth more than that of the new-born.

The general scheme of the growth of the stomach in net weight from early fetal life until full maturity is illustrated by Figure 5. This is a diagram showing the weight of the stomach at various ages, calculated on the basis of the weight of the organ in the new-born. The shaded square on the base line of the diagram represents the weight of the stomach in the new-born, which is considered as one; and the columns to the left and right represent the comparative weight of the organ in fetal and postnatal life.

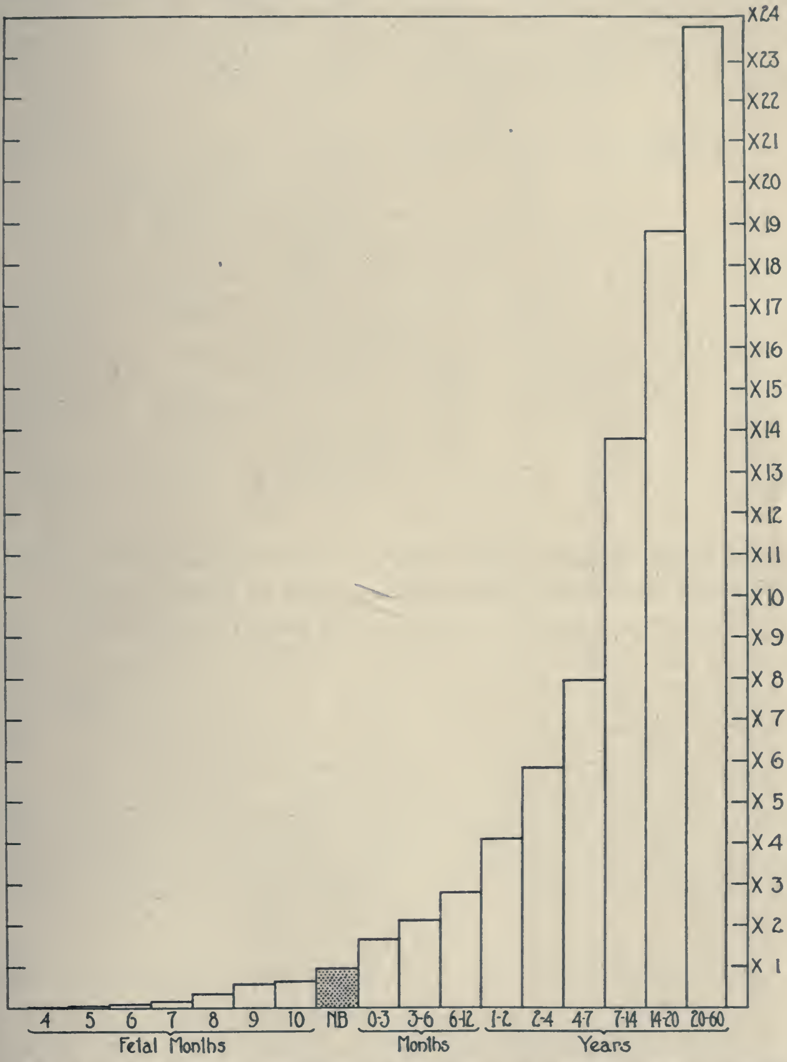


Fig. 5.—A diagram illustrating the weight of the stomach at different periods in fetal and postnatal life as compared to the average weight of the organ in the new-born infant.. The weight of the stomach in the new-born is considered as 1 and is represented by the shaded square in the diagram.

2. THE GROWTH IN SURFACE AREA OF THE GASTRIC MUCOUS MEMBRANE

The data on the growth of the internal surface area of the stomach are scanty. Table 3 gives the available statistics on this subject. The first work on this phase of the stomach's growth was done by Toldt[7] in connection with a study of the development of the gastric glands. He found that the area of the internal surface of the stomach of a fetus of the eighth month was 23.02 square centimeters, and that this area was a little more than doubled in the new-born. At 10 years the area was nearly ten times, at fifteen years about eleven times, and in the adult nearly fifteen times that of the new-born.

TABLE 3.—AREA OF THE INTERNAL SURFACE OF THE STOMACH

Age	Area (in Sq. Cm.) of Internal Surface of Stomach Wall		
	Toldt[7]	Lissenko[8]	Dargien[9]
8th fetal month	23.02	....	....
10th fetal month	53.30	....	....
Birth	52.70	26.9	12.75
2 months	....	60.2	31
3 to 3½ months	....	67.7	48
5 months	....	....	55
6½ to 7 months	....	85.7	67
8 months	....	....	82
9 months	....	125.7	....
11 months	....	....	118
1 year	....	133.9	....
3 years	....	142.9	....
10 years	505.1	....	....
15 years	657.7	....	....
Adult	763.0	454.7	600

Lissenko[8] found the gastric area of the new-born to be approximately 27 square centimeters, or about half as great as Toldt's estimate. This area more than doubled in the first trimester, more than tripled in the second trimester and was increased fivefold by the end of the first year. Between 1 and 3 years there was little growth. The total increase in area between birth and maturity was about seventeenfold.

The most extensive series of observations on the gastric area are those of Dargien.[9] His figures for early infancy are much lower than those of Toldt and Lissenko. In the new-born the average area was 12.75 square centimeters. This was increased nearly four times in

---

7. Toldt, C.: Die Entwicklung und Ausbildung der Drüsen des Magens, Sitzungsber., math.-naturwiss. Cl., k. Akad. Wissensch., Wien., 1881, Section 3, p. 57.

8. Lissenko, W.: The Growth and Structure of the Stomach in Childhood, Dissert. St. Petersburg, 1899. (Quoted from review in Jahresb. f. Anat. **3**: 1900, and from Gundobin, A. P.: Die Besonderheiten des Kindesalters, Berlin, 1912, p. 264 et seq.)

9. Dargien, P.: Surface et volume comparés du l'estomac et du duodenum, Bibliogr. anat. **12**:207, 1899.

the first trimester and five times in the first half year. By the eleventh month the natal area had increased nearly ten times, and by maturity nearly fifty times.

Although considerable differences exist between the figures of Lissenko and Dargien as to the actual area of the gastric mucous membrane, Figure 6 shows that the curves of growth of the area based on these data are of much the same type. In both cases there is a steady and probably fairly regular growth during the first year. Many more data, particularly for the latter part of childhood, are necessary before further conclusions can be drawn on this subject.

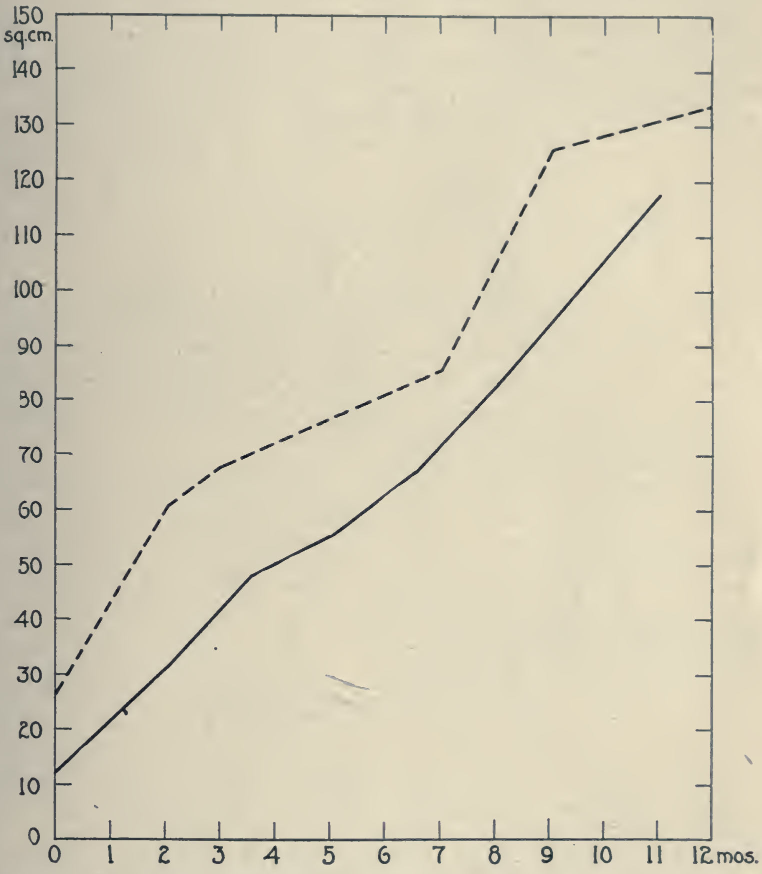


Fig. 6.—Curves illustrating the growth in surface area of the gastric mucous membrane during the first year. Abscissa: area of the gastric mucous membrane in square centimeters; ordinate: age in months. The upper curve (drawn in broken line) is based on the observations of Lissenko.[8] The lower curve is based on the observatoins of Dargien.[9]

3. THE GROWTH OF THE STOMACH IN CUBIC CAPACITY

The growth of the stomach in cubic capacity is obviously much more difficult to determine than either the increase of the organ in weight or the growth of its internal surface area. In the first place, there is no precise criterion of what constitutes a normally filled stomach. Second, no method yet devised of measuring gastric capacity is free from possible error. And third, as has been pointed out by a number of writers, gastric capacity depends not only on the chronologic age of the individual, but on the previous feeding history, the degree of physical development, and the individual physical constitution. In some cases these factors may be more important than that of age. The following material is presented with these points in mind and with the realization that conclusions can be drawn from it only with considerable caution.

The statistics on gastric capacity are very much more extensive than those regarding either stomach weight or gastric surface area. A number of methods have been employed in securing these data, but they may be divided into two general classes — the anatomic and the physiologic. The results secured by these two classes of technic will first be considered separately.

1. *The Anatomic Capacity of the Stomach.*—A number of different methods have been employed for testing the anatomic or postmortem capacity of the stomach. The earlier investigators (Fleischmann,[10] Froloffski[11]) distended the organ with air and dried it in this condition. The cubic capacity of the viscus was then determined by measuring the displacement in water. Drewitz[12] used similarly expanded stomachs, but measured the volume by filling the organ with small glass beads and determining the quantity of these objects required to occupy the entire cavity of the viscus. Most modern investigators have worked with fresh specimens and have measured the capacity by filling the organ with water under various conditions, with an eye to securing its normal expansion. Holt[13] removed the stomach from the body and filled it with water until the rugae were no longer visible when the organ was held before a strong light. Mosenthal,[14] leaving the organ in situ, ligated the pylorus and introduced water through the cardiac orifice at a pressure of about 15 cm.

---

[10] Fleischmann: Klinik der Pädiatrik, Wein., 1875.

[11] Froloffski: Material on the Anatomy of the Digestive Tract in Sucklings, Diss., St. Petersburg, 1876. (Quoted from summaries by Pfaundler, Footnote 2, and from review in Jahresb. f. Anat., 1877.)

[12] Drewitz: Der Saüglingsmagen, Dissert., München., 1891.

[13] Holt, L. E.: Observations on the Capacity of the Stomach in Infancy, Arch. Pediat. **7**:960, 1891; Diseases of Children, New York, Ed. 1, 1897.

[14] Mosenthal, H.: Gastric Capacity of Infants, Arch. Pediat. **36**:261, 1909

until it would hold no more. The organ was then clamped and removed from the body and its contents measured.

Probably the best method for the routine determination of gastric capacity is that devised by Zuccarelli.[15] He removed the organ from the body, distended it with water and then placed it in a vessel of water and opened its orifices. After the pressures of the fluid within and without the organ had adjusted themselves the orifices were again closed, the organ removed from the vessel, and its contents measured. This ingenious method has been extensively employed by a number of French observers, particularly by d'Astros,[16] and by Alliot.[17]

TABLE 4.—A SUMMARY OF OBSERVATIONS ON THE ANATOMIC CAPACITY OF THE STOMACH. (ONLY A PORTION OF PFAUNDLER'S RESULTS ARE CONTAINED IN THE TABLE)

Observer		Fetal Months						Postnatal Weeks								Postnatal Months										Years							
		5th	6th	7th	8th	9th	B.	1st	2nd	3rd	4th	5th	6th	7th	8th	III	IV	V	VI	VII	VIII	IX	X	XI	XII	2	3	4	5	6	7	8	10
Politzer, '58								51-65																		220							
Fleischmann, '75		15				30		46	75	101	77	80						290	260				375										
Stössl, '75							40-50		60-70		70-80	125-145																					
Frolotisky, '76								50	75	105	122	158				167	178	180					253										
Beneke, '80							35-45		153-160			740																					
Drewitz, '90										99	150	115				130	165	253	297	217	289	510	351	535	500	502	588	689	1216				
Holt, '90							34		42		57		74		96	120	132	141	154	168	198	189		238	213	252							
Zuccarelli, '94		7.5	12	13	16	27		37	65	99	45	94	84	85	98	139	139	204		300	190	215		470	399	480	405	510	652	885			960
d'Astros, '94		5-10						59		75		85				147			287							360	500	600					770
Rolch, '95							25	25	40		75					100	107	114		220						300							
Muggia, '96		10					25		70-80			100																					
Pfaundler '97-'98	0 D						8.08				50				51	53	55	57	60	64	69	75	82	90	100	125	140	150	159	167	174	180	
	0 S															75	8	9	10	11	13	16	21	27	36	46	53	59	64	68	71	73	
	15 D																																
	15 S										90				100	110	125	140	160	180	200	225	250	275	290								
	20 D						27				120				140	170	200	230	260	290	325	360	395	430	460	585	680	760	830	890	940	980	
	20 S															140	165	190	215	245	275	305	335	355	370	490	575	640	700	750	800	840	
	30 D						30.8				150				175	200	230	260	295	330	365	405	445	485	515	640	730	810	880	935	970	930	
	30 S															210	235	260	290	325	360	395	430	460	490	620	730	820	890	945	985	1020	
Dargien, '99							34									95	120	140		145	215			240									
Lissenko, '99								35-75				40-140				65-143	68-190																
Fischer, '00											60									255				300									
Alliot, '05			14-17	21-22	24-25	27	35																										
Mosenthal, '09											76	50	74	70		52	68	74		124													

Although Muggia[18] was apparently the first observer to test the capacity of the excised stomach at a definite pressure, by far the most complete investigation of this kind is the well known one of Pfaundler.[19] He measured the capacities of the stomachs of a series of children from birth to 8 years of age at pressures varying from

---

15. Zuccarelli, P.: L'estomac de l'enfant, Thèse Paris, 1894.

16. d'Astros: L'estomac de l'enfant; sa dilatation, Rev. de mal. de l'enf. **12:** 1894.

17. Alliot, L.: Le capacité stomacale du nouveau-né, Thèse Paris, 1905.

18. Muggia, A.: La capacitè e la posizione del ventricolo nei bambini, Giorn. d. r. Accad. di med. di Torino, Series 4, **2:**351, 1896.

19. Pfaundler, M.: Ueber Magencapacität im Kindesalter, Wein. klin. Wchnschr. **10:**961, 1897. Ueber Magencapacität und Gastrectasie im Kindesalter, Biblioth. med. Sec. 4, Part 5, 1898.

0 to 30 cm. of water. Pfaundler concluded that a pressure of about 15 cm. of water produced a distention of the contracted or systolic stomach most comparable with the filled stomach of the living child.

The results of the application of these various methods by different observers are shown in Table 4. An examination of this table shows that while there is much variation in the individual cases, the findings of these several observers, taken as a whole, are in fair agreement, particularly for the period between birth and the eighth or ninth month. Also, if one may judge from the conclusions of Muggia and Pfaundler regarding gastric capacity at different pressures, most investigators, regardless of the method which has been employed, have secured a distention of the stomach equal to or a little greater than that obtained with a pressure of 15 cm. of water.

On the basis of the data presented in Table 4 I have constructed a semidiagrammatic curve illustrating the changes in gastric capacity as determined by all anatomic methods for the period from the fifth fetal to the tenth postnatal month. This curve is shown in Figure 7. An examination of this curve shows that it is divisible into five distinct segments which represent periods in which the increase in gastric capacity, in as far as it may be determined by anatomic methods, differs considerably. Starting at the ~~first~~ 5th fetal month, the capacity of the stomach is approximately 10 c.c. This rises to about 20 c.c. by the middle of the sixth fetal month, but thereafter the increase until the close of prenatal life is rather slow. In the full-term new-born the average capacity is between 30 and 35 c.c. Immediately after birth the curve enters its third phase and shows a rapid rise for about four weeks. At the end of this period the capacity is about 100 c.c., or about three times that of the child at birth. A second period of slow increase follows from the end of the first postnatal month until nearly the close of the third trimester. During this interval the gastric capacity is slightly more than doubled. The actual rate of increase is about 30 per cent. per month, or about one tenth that observed in the first four weeks. At the beginning of the eighth month the curve again begins to rise rapidly. How long this second period of rapid increase continues our data are insufficient to determine, but it is probable that it does not extend far past the end of the first year.

The data on the increase in the anatomic capacity of the stomach in later childhood are less extensive than those regarding the growth in the first year. The figures assembled in Table 4 indicate that the average capacity in the second year is not far from 500 c.c., in the third year about 550 c.c., and in the fourth year about 600 c.c. In the latter half of childhood the average gastric capacity probably lies between 750 and 950 c.c. Apparently there are no data on the capacity of the stomach in adolescence. A large number of observations on the capacity of the adult stomach, as determined by a number of anatomic methods, have been published by Charbrie,[20] Dargien,[9] Legendre,[21] Ost,[22] Pfaundler,[19] Sugari,[23] and others. These studies show great individual variations in the capacity of the organ in maturity, but the more extensive series of observations, such as those of Dargien and Legendre, indicate that the usual and the average capacity of the excised stomach, when moderately distended, lies

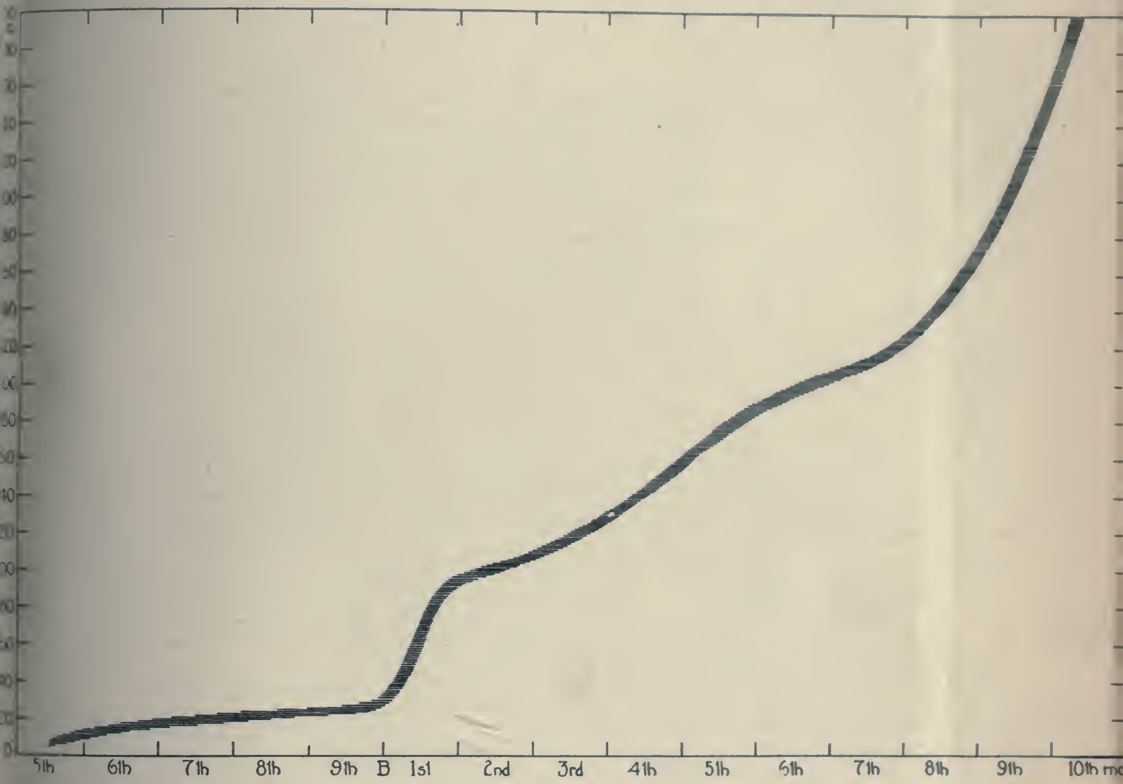


Fig. 7.—A semidiagrammatic curve illustrating the increase of gastric capacity during fetal life and in the first year as determined by all anatomic methods. Abscissa: age in fetal or postnatal months; ordinate: capacity of the stomach in cubic centimeters. Based on the material presented in Table 4 (with the exception of the data of Pfaundler).

---

[20] Charbrie: Thèse Toulouse, 1894.

[21] Legendre: Thèse Paris, 1886.

[22] Ost, A.: Beiträge zur Bestimmung der Capacität des Magens, Dissert. Dorpat, 1891.

[23] Sugari, T.: Ueber die Lage und Kapazität des Magens bei Japanern, Centralbl. f. allg. Path. **20**:390.

between 1,200 and 1,600 c.c. The figures of Pfaundler on the capacity of the stomach at all pressures are much above those just quoted, but his observations were made on diastolic stomachs. Accepting the figures offered in the foregoing, it appears that the increase in the anatomic capacity of the stomach between birth and maturity is forty or fifty-fold, or about twice as much as the increase of the organ in weight.

2. *The Physiologic Capacity of the Stomach.*—Two methods have been employed in the study of gastric capacity in the living. The first and usual one is to determine the amount of food ingested by weighing the child before and after feeding; and the second is to measure the amount of air introduced into the stomach at a definite pressure by means of a tube connected with a cylinder which may be raised and lowered into a vessel of water. As the cylinder is graduated it is possible to determine how much air is driven from it into the stomach at a given pressure. The second method, which was devised by Jaworski[24] and Kelling,[25] has been developed and applied to the study of gastric capacity in children by Pfaundler.[19] While the results obtained by this method are of great interest, they are hardly extensive or conclusive enough to warrant their graphic analysis at present. The familiar method of determining gastric capacity by weighing the child before and after feeding was apparently first systematically employed by Guillot,[26] although it is difficult to believe that such an obvious procedure was not in use long before this time. Since this date an enormous amount of data obtained by this method has been published. However, much of the earlier work in this field is of no great value because of the short feeding intervals employed by the first observers.

The findings regarding the capacity of the stomach as determined by the method of weighing before and after feeding may be divided into two groups. The first, and by far the most extensive, are those of the average amount of food ingested at a single feeding. The second, of which there are comparatively few published records, are of the maximum amount of food ingested at a single feeding in a given period. The former have by far the greater practical importance, but as Feer[27] and others have pointed out, the maximum feeding is probably a better index of possible gastric capacity.

---

[24] Jaworski, W.: Ueber Anwendung der Gase für therapeutische und diagnostische Zwecke bei Magenkrankheiten, Deutsch. Arch. f. klin. Med. **35**:79, 1884.

[25] Kelling, G.: Ein einfaches Verfahren zur Betimmung der Magengrosse mittels Lust, Deutsch. med. Wchnschr. **18**:260 and 1191, 1892.

[26] Guillot, N.: De la nourrice et du nourrisson, Union méd., 1852, p. 61.

[27] Feer, E.: Beobachtungen über die Nahrungsmengen von Brustkindern, Jahrb. f. Kinderh. **42**:195, 1896.

Some of the better known determinations of the average capacity as found by the average feeding method are shown in graphic form in Figure 11. These will be considered in detail in another part of this article, but it may be pointed out at this time that the curves based on these determinations are all of practically the same type and that their divergences are probably due to the nature of the material and slight differences in method employed by the various observers.

Apparently, the first data on the maximum capacity of the stomach in infancy were published by Ahlfeld[28] in 1878. This author bases his tables on the maximum feeding in each weekly period of the time in which his cases were under observation. Later workers have followed Ahlfeld in the use of this time unit in determining the maximum physiologic capacity. More material of this kind was published by Haehner[29] and by Feer.[27] The latter published a table giving the average maximum capacity of seven cases, three of his own and four observed by Ahlfeld and Haehner. Feer's summary is the only one of the kind which has been published.

A considerable number of observations on the maximum physiologic capacity of the stomach have appeared in the literature since Feer published his summary, and a complete list of the published feeding histories of children which extend over a number of weeks and include a record of the amount of food ingested at each meal would now probably contain over twenty cases. From the available data of this kind I have selected eleven cases and have prepared a summary of the average and maximum feedings for each week from birth to the close of the seventh month. This list has been limited to children having at least an average birth weight, who have shown a normal gain in weight during the period of observation, and who, for the greater part of the period at least, were breast fed. This summary is given in Tables 5 and 6, and the graphs shown in Figures 8, 9 and 10 are based on this material. Brief histories of these eleven cases are given:

REPORT CF CASES

Histories of eleven children whose feeding records are summarized in Tables 5 and 6, and are shown in graphic form in Figures 8, 9 and 10.

Case 1.—(Selter.[30]) Male; birthweight, 3,250 gm.; birthweight recovered on thirteenth day; weight at end of fourth week, 3,840 gm.; eighth week, 4,800 gm.; tenth week, 5,310 gm.; sixteenth week, 6,390 gm.; nineteenth week,

---

[28] Ahlfeld, F.: Ernährung des Saüglings, Leipzig, 1878.

[29] Haehner, H., and Pfeiffer: Festschr. f. E. Henoch, 1890, p. 90 et seq.

[30] Selter, P.: Nahrungsmengen und Stoffwechsel des normalen Brustkindes, Arch. f. Kinderh. **37**:91, 1903.

6,980 gm.; seven feedings per day; breast feeding throughout period of observation.

Case 2.—(Paffenholz.[31]) Female; first child; birthweight, 3,430 gm.; birthweight regained on twenty-seventh day; weight at end of fourth week, 3,490 gm.; eighth week, 4,590 gm.; twelfth week, 5,520 gm.; sixteenth week, 6,490 gm.; twentieth week, 7,290 gm.; six or seven feedings per day; breast feeding until ninetieth day; thereafter mixed feeding.

Case 3.—(Paffenholz.[31]) Male; second child; birthweight, 4,140 gm.; birthweight regained on tenth day. Weight at end of fourth week, 4,710 gm.; eighth week, 5,680 gm.; twelfth week, 6,520 gm.; sixteenth week, 7,140 gm.; twentieth week, 7,530 gm.; six feedings per day until middle of fourteenth week; five feedings per day until middle of sixteenth week; four feedings per day thereafter; breast feeding until end of thirteenth week; mixed feeding thereafter.

Case 4.—(Feer.[27]) Male; second child; birthweight, 3,615 gm.; birthweight regained before end of third week; weight at end of fourth week, 4,165 gm.; eighth week, 5,369 gm.; twelfth week, 6,153 gm.; sixteenth week, 6,864 gm.; twentieth week, 7,399 gm.; twenty-fourth week, 7,748 gm.; twenty-eighth week, 8,022 gm.; breast feeding until 216th day; thereafter mixed feeding; seven feedings per day for first two weeks; thereafter five, six or seven feedings.

Case 5.—(Feer.[27]) Female; first-born; birthweight, 3,765 gm.; birthweight regained in fifth week; weight at end of fourth week, 3,780 gm.; eighth week, 4,525 gm.; twelfth week, 5,160 gm.; sixteenth week, 5,550 gm.; twentieth week, 6,030 gm.

Case 6.—(Haehner.[32]) Male; second-born; birthweight, 2,950 gm.; birthweight regained seventh day; weight end of fourth week, 3,580 gm.; eighth week, 4,505 gm.; eleventh week, 5,045 gm.; feedings rather irregular; generally seven per day until end of eighth week, although on some occasions nine per day, and on some five; ninth week and thereafter generally six feedings per day; breast feeding throughout.

Case 7.—(Haehner[33]) Female; first-born; birthweight, 3,100 gm.; birthweight regained on ninth day; weight at end of fourth week, 3,607 gm.; eighth week, 4,793 gm.; twelfth week, 5,636 gm.; twentieth week, 6,370 gm.; twenty-fourth week, 5,650 gm.; twenty-eighth week, 7,300 gm.; thirty-second week, 7,830 gm.; generally five feedings per day, although sometimes four or six, until close of eighteenth week; thereafter generally four feedings, although five not unusual; breast feeding until close of twenty-third week; thereafter mixed feeding.

Case 8.—(Selter.[30]) Male; second child (?); birthweight, 2,870 gm.; birthweight regained on tenth day; weight at end of fourth week, 3,580 gm.; sixth week, 4,030 gm.; tenth week, 4,760 gm.; thirteenth week, 5,250 gm.; twentieth week, 6,120 gm.; six feedings, or occasionally five per day; breast feeding throughout.

Case 9.—(Biermer.[34]) Sex and parity not stated; birthweight, 3,850 gm.; weight at end of second week, 3,830 gm.; fourth week, 3,950 gm.; eighth week, 4,420 gm.; twelfth week, 4,880 gm.; sixteenth week, 5,550 gm.; twentieth week, 6,440 gm.; twenty-fourth week, 7,090 gm.; twenty-eighth week, 7,710 gm.; thirty-second week, 8,330 gm.; five feedings per day throughout period; breast feeding until twenty-sixth week; thereafter mixed feeding.

---

31. Paffenholz: Beitrag zur Kenntniss der Nahrungsmengen natürlichen ernahrten Sauglinge, Arch. f. Kinderh. **37**:104, 1903.

32. Weitere Beobachtungen über die Nahrungsaufnahme des Kindes und das Wachsthum in ersten Lebensjahre, Jahrb. f. Kinderh. **21**:289, 1884.

33. Haehner, H.: Ueber die Nahrungsaufnahme des Kindes an der Mutterbrust und das Wachsthum in ersten Lebensjahre, Jahrb. f. Kinderh. **15**:23, 1880.

34. Biermer, R.: Beiträge zur Frage der naturlichen Ernahrung, Arch. f. Kinderh. **47**:68. 1908.

Case 10.—(Biermer.[34]) Parity and sex of child not stated; birthweight, 3,750 gm.; birthweight regained at end of third week; weight at end of fourth week, 3,890 gm.; eighth week, 4,250 gm.; twelfth week, 4,710 gm.; sixteenth week, 5,085 gm.; twentieth week, 5,700 gm.; twenty-fourth week, 6,310 gm.; twenty-eighth week, 6,830 gm.; thirty-second week, 7,107 gm.; five feedings throughout period; length of feeding, ten minutes; breast feeding until end of thirty-first week.

Case 11.—(Ahlfeld.[28]) Weight at end of fourth week, 3,620 gm.; eighth week, 4,400 gm.; twelfth week, 5,170 gm.; sixteenth week, 6,220 gm.; twentieth week, 6,957 gm., 7,700 gm., 8,325 gm.; thirtieth week, 8,580 gm.

TABLE 5.—Physiologic Capacity of the Stomach as Determined from the Amount of the Average Meal in Eleven Selected Cases

Weeks	Capacities in Individual Cases (in grams*)											No. of Cases per Week	Average Capacity in All Cases, Gm.
	1	2	3	4	5	6	7	8	9	10	11		
1st	...	...	52	66	73	43	51	50	..	62	...	7	57
2d	74	...	95	81	89	66	67	70	...	111	...	8	82
3d	79	...	116	98	80	68	77	91	113	125	...	9	94
4th	87	73	131	122	88	81	94	102	130	131	104	11	104
5th	89	92	118	...	108	91	113	109	139	134	128	10	112
6th	99	102	127	...	111	98	144	117	150	133	150	10	123
7th	108	107	146	...	133	101	157	...	166	138	157	9	135
8th	116	119	145	...	132	109	162	...	152	131	163	9	136
9th	115	131	144	...	127	101	153	...	132	143	167	9	135
10th	120	144	162	...	132	98	159	131	126	152	164	10	138
11th	...	142	153	...	122	114	153	...	151	148	162	8	145
12th	...	145	155	...	126	...	171	...	168	148	173	7	155
13th	...	136	155	...	125	...	168	122	176	149	200	8	154
14th	...	151	153	...	122	...	175	..	179	151	200	7	161
15th	...	158	197	...	118	...	183	...	188	159	225	7	176
16th	123	159	...	...	110	...	156	...	181	157	212	7	157
17th	138	170	...	...	109	...	150	...	197	166	208	7	162
18th	161	169	...	...	109	...	176	...	190	162	241	7	171
19th	139	172	...	...	...	..	207	...	193	166	231	6	184
20th	...	173	...	...	...	...	198	115	202	166	212	6	178
21st	...	...	...	...	...	...	196	...	198	173	233	4	200
22d	...	...	...	...	...	...	190	...	197	173	200	4	190
23d	...	...	...	...	...	...	184	...	201	176	217	4	195
24th	...	...	...	...	...	...	157	...	199	178	214	4	187
25th	...	...	...	...	...	...	169	...	199	185	205	4	189
26th	...	...	...	...	...	...	188	...	205	179	207	4	195
27th	...	...	...	...	...	...	199	...	200	191	224	4	204
28th	...	...	...	...	...	...	219	...	208	198	215	4	210
29th	...	...	...	...	...	...	215	...	206	189	261	4	217
30th	...	...	...	...	...	...	220	...	197	182	263	4	216

* The cases are numbered in accord with the protocols given on pages 411 and 412.

The changes in the average intake per meal for each week from birth to 14 weeks in these cases is shown in graphic form in Figure 8. Figure 9 is a similar graph illustrating the changes in the maximum meal in the given week in the same cases. Both of these graphs illustrate the great individual variation to be expected in measurements of this kind, even when the material is limited to apparently healthy children having a normal birth weight. The curves as a whole, however, tend to gather in a fairly compact cluster, the upper and lower boundaries of which are rather regular. An examination of the protocols shows no particular relation between the weight of the child and the character of its feeding curve. Furthermore, there seems to be no close relation between the weekly average and the weekly maximum meal in the individual cases.

The curves shown in Figure 10 illustrate the average intake per single meal in all of the cases of the series and maximum single intake in a given week in all of these cases. The curves have been slightly smoothed by inspection. Both of these curves are of the same type. Both show a rapid rise in the first three weeks, and thereafter a much slower, but a constant elevation until the end of the period of observation. The values of the upper curve, of the maximum intake, are, however, from 40 to 65 per cent. higher than those of the lower curve of average intake. We may conclude, therefore, in so far as it is possible to judge at all of gastric capacity from this type of observation, that the average or usual expansion of the stomach at a single meal in the first half year of life represents approximately two-thirds of the physiologic capacity of the organ, and that the average and the maximum physiologic capacity continue to grow at about the same rate during this period.

TABLE 6.—MAXIMUM PHYSIOLOGIC CAPACITY OF THE STOMACH AS DETERMINED FROM THE MAXIMUM MEAL OF EACH WEEK IN ELEVEN SELECTED CASES

Weeks	Capacities in Individual Cases (in grams*)											No. of Cases per Week	Average Maximum Capacity in All Cases
	1	2	3	4	5	6	7	8	9	10	11		
1st	...	45	120	120	125	85	124	110	...	120	...	8	106
2d	110	70	130	125	160	100	113	120	...	170	..	9	122
3d	110	100	160	145	135	125	170	140	170	180	...	10	143
4th	130	120	170	165	155	130	163	170	180	190	140	11	155
5th	130	140	170	190	220	160	187	250	200	200	160	11	183
6th	145	150	190	220	180	160	230	205	210	200	200	11	190
7th	180	150	230	195	200	160	218	...	250	210	210	10	200
8th	200	170	220	205	260	160	250	...	210	225	245	10	214
9th	180	195	220	205	190	180	255	...	180	240	250	10	209
10th	180	210	240	240	205	170	230	240	170	220	225	11	211
11th	...	180	225	230	190	170	245	...	240	230	245	9	217
12th	...	190	220	285	195	...	280	...	230	230	275	8	238
13th	...	200	220	280	190	...	290	250	270	240	350	9	254
14th	...	220	230	295	195	...	285	...	280	230	320	8	257
15th	...	210	250	290	190	...	280	...	270	230	300	8	245
16th	190	210	280	330	175	...	285	...	300	260	315	9	261
17th	270	230	290	300	170	...	260	...	340	250	320	9	270
18th	230	215	310	300	170	...	265	...	350	250	340	9	270
19th	210	250	280	320	...	...	290	...	310	260	315	8	279
20th	...	250	280	290	...	...	305	230	310	220	325	8	277
21st	...	...	...	310	...	...	310	...	360	250	335	5	313
22d	...	...	...	310	...	...	330	...	350	250	296	5	307
23d	...	...	...	290	...	...	320	...	340	260	350	5	312
24th	...	...	...	260	...	...	340	...	330	270	330	5	306
25th	...	...	...	300	...	...	270	...	330	260	290	5	290
26th	..	...	...	265	...	...	300	...	320	260	300	5	306
27th	...	...	...	275	...	...	300	...	310	280	345	5	302
28th	..	...	...	270	...	...	340	...	320	280	430	5	330
29th	..	...	...	355	...	...	275	...	320	250	400	5	322
30th	..	...	...	300	...	...	275	...	310	250	350	5	299

* The cases are numbered in accord with the protocols given on pages 411 and 412.

3. *A Comparison of the Findings Obtained by Anatomic and Physiologic Measures of Gastric Capacity.*—That many objections can be raised to the accuracy of the results obtained by either anatomic or physiologic measures of gastric capacity is very evident. As for the

former, they are applicable only to material which is often pathologic and of which the history is often incompletely known. The results might be expected to depend to a large extent on the condition of the stomach wall at the time of death and on the subsequent changes which it has undergone. There is, furthermore, considerable evidence to show that the filling of the stomach in life is not a simple expansion of the organ following the pressure of its contents, such as is obtained in distending the organ postmortem by artificial means. The method of weighing the child before and after feeding is also open to criticism as a test of actual gastric capacity. The results are dependent to some extent both on the amount of milk available in the mammary gland of the mother, in cases of breast feeding, and on the infant's appetite.

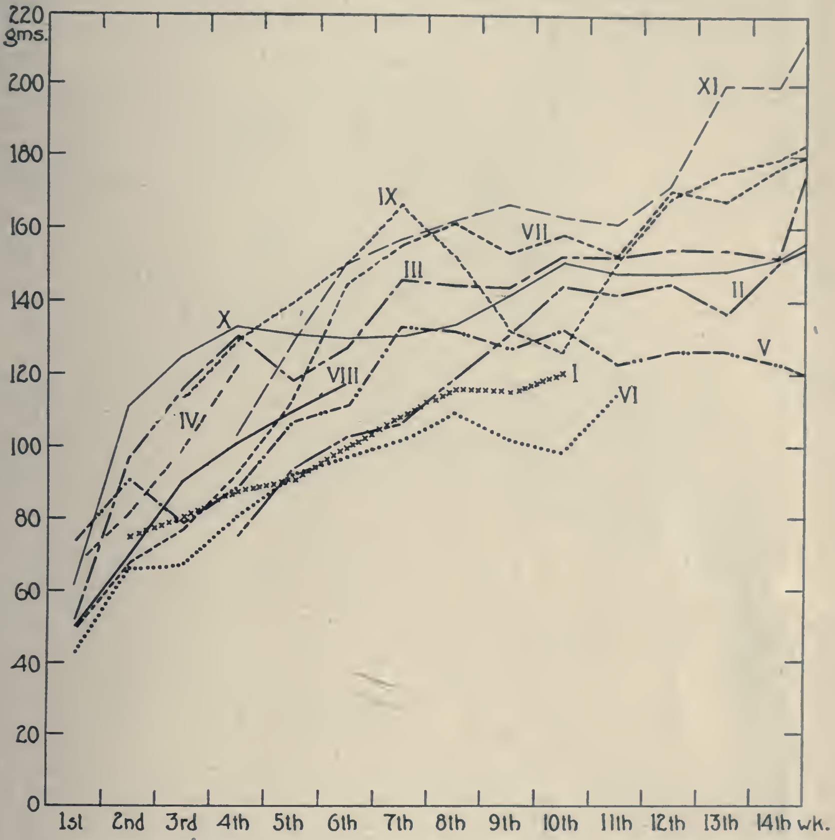


Fig. 8.—A series of curves showing the gastric capacity as determined by the average intake per single meal per week in the eleven selected cases on which the average curves shown in Figure 10 are based. The curves of the individual cases are numbered in correspondence with the protocols given on pages 411 and 412.

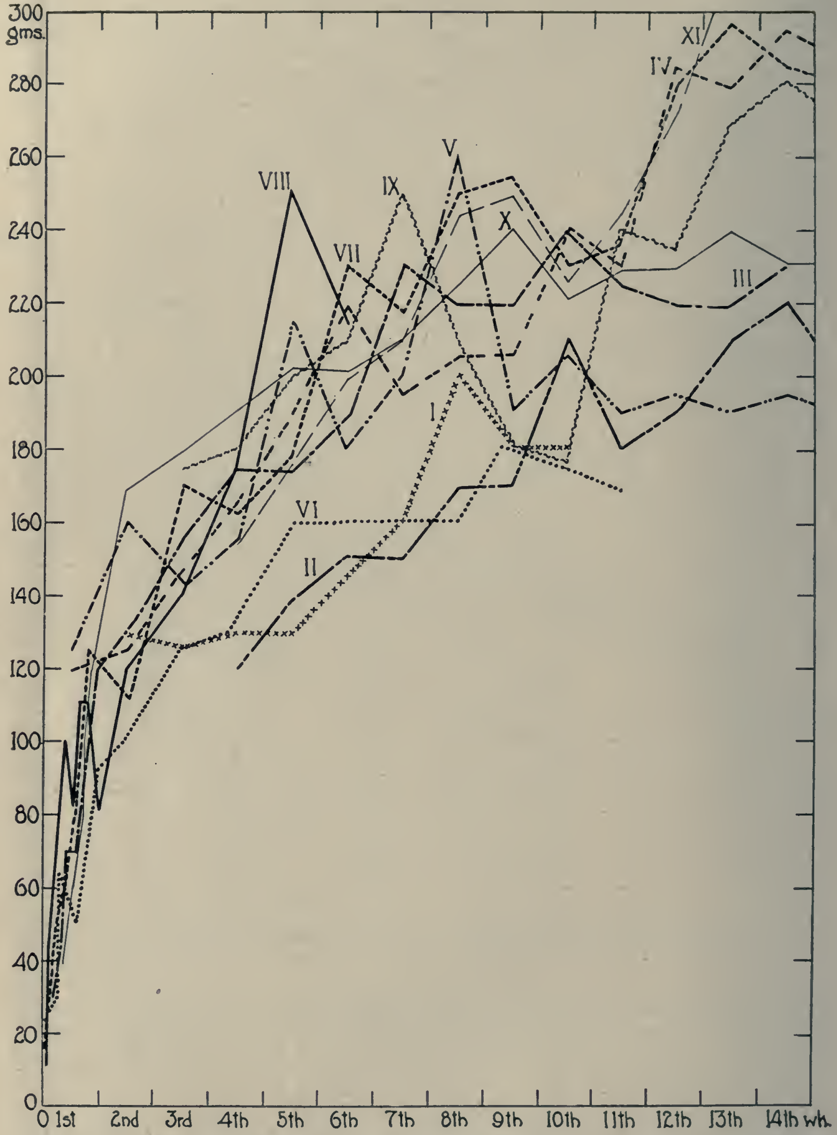


Fig. 9.—A series of curves illustrating the growth in gastric capacity as determined by the maximum single meal per week in each of the eleven selected cases, which have been combined to determine the average curve shown in Figure 8. The curves are numbered in correspondence with the cases in the protocols.

Again the roentgenographic studies of Levin and Barret,[1] Ladd,[35] Pisek and Lewald,[36] DeBuys and Henriques,[37] and others, have shown, first, that food is expelled through the pylorus very shortly after its ingestion, and secondly, that the rate of this expulsion is greatly affected, even in normal infants, by such factors as the nature of the food, posture of the body, etc. The early passage of food from the stomach will lead to an overestimate of gastric capacity by the feeding

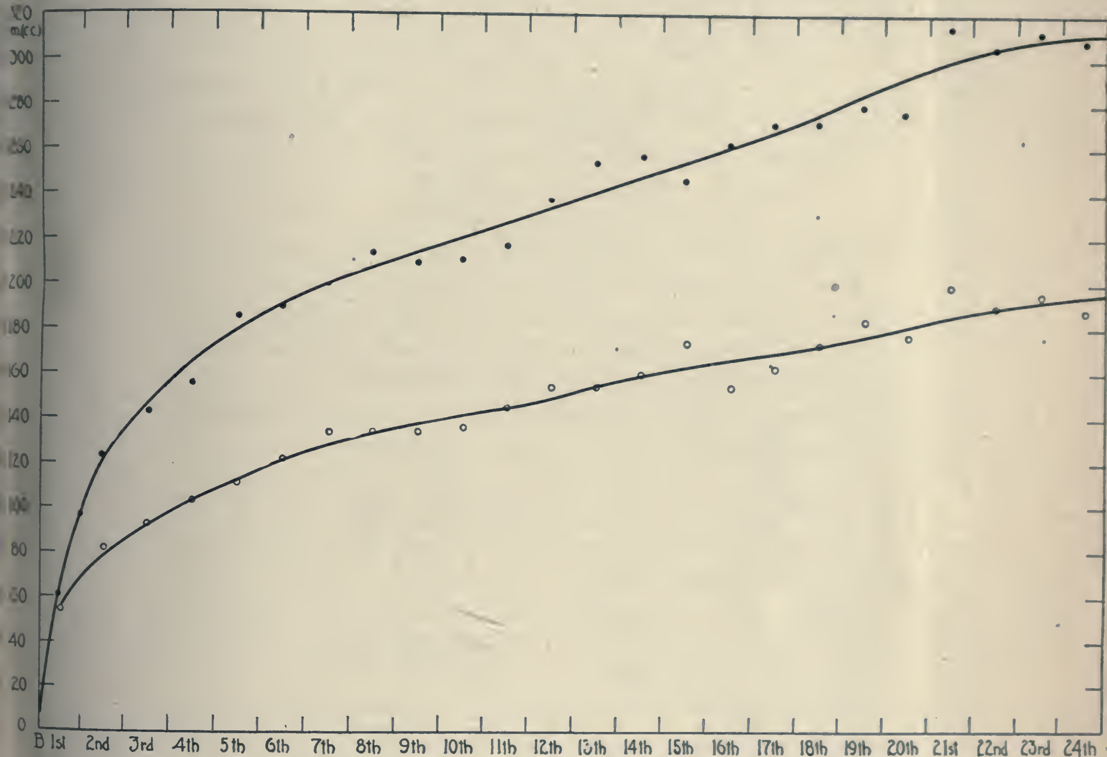


Fig. 10.—Two curves illustrating the increase of gastric capacity during the first six months as determined from the feeding records of the eleven selected cases on pages 411 and 412. The curves have been smoothed by inspection. The average points are indicated by dots or small circles. The upper curve is of the average maximum single meal in the week indicated. The lower curve is of the average intake per single meal in the week indicated. The segments of the curves falling in the period of the first week are based on the average and the maximum meal per day in so far as the data permitted.

---

35. Ladd, M.: The Influence of Variations of Diet on the Gastric Motility of Infants, Tr. Am. Pediat. Soc. **25**:81, 1912.

36. Pisek, G. R., and LeWald, L. T.: The Further Study of the Anatomy and Physiology of the Infant's Stomach Based on Serial Roentgenograms, Tr. Am. Pediat. Soc. **25**:150, 1913.

37. DeBuys, L. R., and Henriques, A.: Effect of Body Posture on the Position and Emptying Time of the Stomach, Am. J. Dis. Child. **15**:190, 1918.

method. On the other hand, the presence of the cardiac gas bubble and the loss of moisture from the lungs and skin during the feeding period will lead to an underestimate of the actual capacity of the organ. While these two groups of factors may in part balance one another, they are all variable to a degree.

Since evidently the present physiologic and anatomic methods can give but approximations of the actual gastric capacity, a comparison of their results is desirable in order to determine what correlation, if any, exists between them, and to eliminate possibly some of the sources of error. Such a comparison is particularly important because the application of the physiologic method of determining gastric capacity by measuring the amounts of single feedings is limited to a comparatively short period in infancy, and at present our knowledge of the growth of the capacity of the stomach in fetal life and in later childhood must rest on data secured by anatomic methods.

Two important studies of the relation of anatomic to physiologic capacity have been published. Pfaundler[19] tested the gastric capacity of a number of living children by his method of expanding the stomach with air at a known pressure, and also determined the postmortem gastric capacities of the same children by distending the stomachs in situ with water at a definite pressure. The number of normal cases studied in this way was rather limited. The results show a fair degree of correlation, although the anatomic capacity varied considerably in different cases, being sometimes greater and sometimes less than the capacity at the same pressure in the living. Pfaundler also compared the averaged results of his findings on the capacity of excised stomachs with other authors' averages of the capacity as determined by the feeding method. He pointed out that his data on the capacity of the excised stomach at a pressure of 30 cm. of water were in substantial agreement with Freer's[27] figures on the average maximum feeding capacity. He also compared his figures of the capacity of the stomach at a pressure of 15 cm. of water with Escherich's table of the amounts of average single feedings and found that the data obtained by the two methods were in fair agreement, although the anatomic figures were slightly lower in the first six months and higher in the last six months of the first year than were the physiologic.

Mosenthal[14] compared the physiologic capacities of twenty-four children with the anatomic capacities in the same cases. All of these children were fed on cow's milk at three-hour intervals. Their ages varied from 4 to 35 weeks. The anatomic capacities were determined by filling the stomach with water in situ at a pressure of 15 cm. Mosenthal found that in all instances, with the exception of children with diastolic stomachs, the living child ingested more fluid than its stomach would hold postmortem. The average amount of milk taken at each feeding was 3.6 ounces and the average postmortem capacity was 2.6 ounces. Mosenthal therefore concluded that the physiologic capacity of the stomach was considerably greater than the capacity as determined by anatomic methods.

A further test of the relation between anatomic and physiologic capacity may be made by comparing graphically the results which have been obtained with the different modifications of the two methods. Such a comparison is shown in Figure 11, which contains a series of curves already presented in this paper, together with a number of curves based on some of the better known estimates of gastric capacity as determined by the feeding method. These curves are all drawn to a uniform scale.

Examining this graph it will be seen that the curves fall quite definitely into an upper and a lower group: The upper group is formed by the curve of the maximum feeding capacity, as determined from selected cases, and the curves based on Pfaundler's data on the capacity of the excised stomach at a pressure of 30 cm. of water (Curves I, III and IV, Fig. 11). All of the remaining curves gather closely together at a somewhat lower level in the first part of their course. They are all very similar for the segments representing the growth of capacity in the first six months and the maximum variation between them is only about 40 c.c. The curve based on the average intake per single feeding as determined from the eleven selected cases (Curve VII) is the highest of the group in the first segment of the graph, but this is later exceeded by the curve of anatomic capacity at a pressure of 20 cm. of water and by the curve of capacity as determined by all anatomic methods (Curves V and VI). The lowest curves for this period are first the one based on Holt's (X) figures for the average feeding, and later the curve based on Pfaundler's data on the capacity of the excised stomach at 15 cm. water pressure (Curve XIII).

The later part of the second group of curves, representing gastric capacity from six months to one year, do not show the close correspondence observed in the earlier period. The curve based on anatomic capacity as determined by all methods (Curve V) rises very rapidly after the seventh month. That based on Pfaundler's data on the capacity of the excised stomach at a pressure of 20 cm. of water also shows a rapid rise at about this period. The curves based on the feeding observations of Rotch,[38] (IX) Ladd[39] (VIII) and Holt (X),

---

[38] Rotch, T. M.: Pediatrics, Philadelphia. Ed. 1. 1896. p. 79 et. seq.

[39] Ladd, M.: Percentage Modification of Milk in Infant Feeding, Boston M. & S. J. **198:**5, 1903. Studies in the Nutrition and Digestion of Infants, Arch. Pediat. **30:**740, 1912.

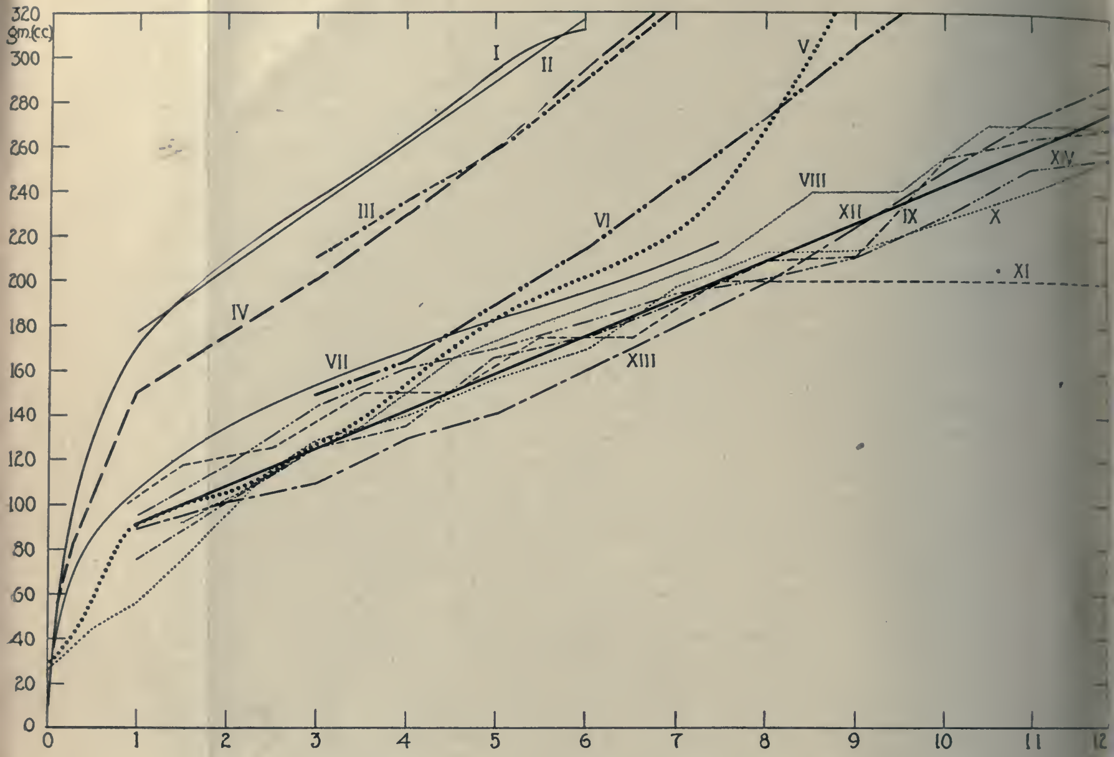


Fig. 11.—A series of curves illustrating the increase in gastric capacity in the first year as determined by a number of different methods, and also two curves based on empirical formula: 1. Curve of average maximum feeding per week in selected cases (corresponding to the upper curve in Figure 8); 2. Curve based on the formula $C = 150 + 7N$ where C is the gastric capacity in cubic centimeters or grams and N is the age in weeks. 3. Curve of capacity of excised systolic stomachs at a pressure of 30 cm. of water (Pfaundler's data). 4. Curve of capacity of excised diastolic stomachs at a pressure of 30 cm. of water (Pfaundler's data). 5. Curve of gastric capacity as determined from the data of all anatomic methods excepting Pfaundler's. 6. Curve of the capacity of the excised stomach at a pressure of 20 cm. of water (Pfaundler's data). 7. Curve of the average intake per single feeding in the given week in selected cases (corresponding to the lower curve in Figure 8 but extended over a somewhat longer period). 8. Curve of the average amount of a single feeding as determined by Ladd.[39] 9. Curve of the average amount of a single feeding as determined by Rotch.[38] 10. Curve of the average amount of a single feeding as determined by Holt. 11. Curve of the average amount of a single feeding as determined by Escherich.[40] 12. Curve based on the formula $C = 75 + 4N$ where C is the capacity in cubic centimeters or grams and N the age in weeks. 13. Curve of capacity of excised systolic stomachs at a water pressure of 15 cm. (Pfaundler's data). 14. Curve of the average amount of a single feeding as given in Smith's[41] feeding chart. The curve has been enlarged to the same scale as the other curves in this figure.

---

40. Escherich: (Quoted from Pfaundler, 19.)

41. Smith, C. H.: A Graphic Feeding Chart: A Method for Calculating Milk Percentages and Caloric Values, Arch. Pediat. **29**:827, 1912.

on Smith's feeding chart (XIV), and on Pfaundler's figures of anatomic capacity at 15 cm. water pressure, continue their former course until the end of the first year while the graph based on Escherich's[40] estimate of the average intake per single meal falls somewhat lower (Curve XI).

On the basis of this general graph several conclusions can be drawn as to the results obtained with the various methods of determining gastric capacity in the first year. These may be summarized as follows:

1. Most curves of gastric capacity, regardless of the method by which this capacity is determined, are of the same general type. They show an initial stage of rapid rise followed by a much longer stage in which the rise is slower and without important changes in rate. The initial stage of rapid rise extends from birth to the end of the third or fourth week. The second stage extends from the latter time to the end of the first year. The exceptions to this rule are the curve based upon Holt's observations in which the period of the initial rise is somewhat longer than usual, and the curve based on the average anatomic capacity as determined by all methods which rises very rapidly after the seventh month. The rapid rise of the latter curve is probably due to the inclusion in the material on which it is based of a number of pathologic and diastolic stomachs.

2. The curve of gastric capacity as determined from the maximum meal in a given week corresponds to a curve of the postmortem capacity of the stomach at a pressure at a little over 30 cm. of water. The capacity as determined by this method is from 50 to 75 per cent. greater than the capacity as determined by the majority of other methods.

3. For a period beginning shortly after birth and extending to the close of the fifth or sixth month, the curve of the average anatomic capacity as determined by all methods is in substantial agreement with the curves based on some of the more extensive collections of data on the amount of the average single feeding. While it is a little higher than some of these, it is lower than the curve based on the average single feeding in the eleven selected cases considered in this article.

4. All curves based on the average intake at a single meal fall between the curves of anatomic capacity at 15 cm. and at 20 cm. water pressure, for the period from 2 to 8 months. Thereafter they tend to fall below the curve of anatomic capacity at 15 cm. water pressure.

5. Since most of the curves of the increase in gastric capacity for the period between the end of the third or fourth week and the close of the first year tend to approach straight lines, it is possible to estab-

lish certain empirical formulas of a simple type which will represent them fairly well. Such formulas are based on a constant initial sum, with a regular weekly or monthly increment. A curve which, for the period under consideration, will pass through the middle of the group of curves representing the average intake per single meal, the average anatomic capacity for the first six months, and the curve based on Pfaundler's data on the anatomic capacity of the stomach at 15 cm. water pressure, is represented by the formula $C = 75 + 4N$, where C is the gastric capacity in grams or cubic centimeters and N is the age in weeks. A curve which will closely approach the curve of maximum meal per week is indicated by the formula $C = 150 + 7N$. This formula holds good from the fourth to the twenty-fourth week. A curve which will closely follow the curve based on the average single feeding in selected cases from the fourth week to the middle of the eighth month is represented by formula $C = 90 + 5N$.

I wish to express my thanks to Drs. J. P. Sedgwick and C. M. Jackson for valuable suggestions in connection with this study.

Reprinted from the American Journal of Diseases of Children
June, 1919, Vol. XVII, pp. 395-422

American Medical Association
Five Hundred and Thirty-Five North Dearborn Street
CHICAGO

760.17.

165/

Reprinted from THE ANATOMICAL RECORD,
Vol. 15, No. 4, November, 1918

ON THE TIME OF THE POST-NATAL OBLITERATION OF THE FETAL BLOOD-PASSAGES (FORAMEN OVALE, DUCTUS ARTERIOSUS, DUCTUS VENOSUS)

RICHARD E. SCAMMON AND EDGAR H. NORRIS

Institute of Anatomy, University of Minnesota

THREE FIGURES

It is generally recognized that two separate processes are involved in the occlusion of the fetal blood passages after birth. The first is the simple functional closure which takes place, in the great majority of cases, at or immediately following birth; the second is the permanent anatomic obliteration which occurs at a later period. The mechanics and histology of the latter process are generally discussed in our larger treatises on obstetrics and pediatrics, and in our major anatomical texts; but the time of postnatal obliteration is often unmentioned, although in a number the statement is made that the obliteration takes place in the first few days or, at most, weeks after birth.

The origin of the current concepts as expressed in these larger texts can be traced, we think, to the first statistical study on this subject, which was published ninety years ago by the French clinician Billard. This investigator collected data on the obliteration of the ductus venosus, ductus arteriosus, and foramen ovale in a series of one hundred and twenty-eight children who died in the first eight days of life. He found instances of the obliteration of all these passages on the first day after birth. The foramen ovale and ductus arteriosus were closed in over fifty per cent of his cases on the eighth day while the ductus venosus was closed in a still larger number. He therefore concluded that the obliteration of the fetal blood-passages proceeded very rapidly in the first few days of life—an opinion in accord with that held by a number of writers in the eighteenth century. The results of Billard's study were published in his 'Traité des maladies des enfants nouveau-nés' in 1828. This work was extremely popular in its time; it passed through a number of French editions, was translated into Italian and German and twice appeared in American editions. In several publications of the middle of the last century Billard's figures are cited and his name is quoted in connection with them. In later works, however, the same opinion has been repeatedly expressed but its source apparently has been forgotten.

A large amount of data concerning the chronology of the postnatal obliteration of the fetal blood-passages has accumulated since the time of Billard. This material is scattered through the periodical literature of legal medicine, obstetrics, and pediatrics and is also included in a number of rather inaccessible brochures. In the following pages we have collected as much as possible of these scattered data and have arranged them in tabular and graphic form. In so doing we have confined ourselves to those records in which series of consecutive cases have been assembled. These records include observations on children who were born at term, and also a few cases of children who were prematurely born. In most instances, however, investigators have failed to separate these two groups. A comparison of the records of the few known cases of prematurity with those of children known to be born at term shows no appreciable difference in the time of obliteration of the fetal blood-passages. We have therefore combined them in our tables. In all cases the patency of the fetal passages was tested either by injection or by probing, excepting those of Faber ('09) which were examined microscopically.

The results made apparent by this combined series of observations are at variance with the current concepts on the subject as expressed in most of our larger texts, and also with the results of Billard. Billard's observations were confirmed by Bernutz ('65) who found the ductus arteriosus closed in fourteen cases in a series of twenty-one children who died between the tenth and twentieth days, and in thirty-six out of thirty-eight children dying between the twentieth and sixtieth days. Since this time no observer has substantiated these findings, although a number of series much larger than those of Billard and Bernutz[1] have been collected. Thus Elsässer ('52), in a series of nearly three hundred observations upon children of the first month, found obliteration of the ductus arteriosus in about two per cent and of the foramen ovale in about three per cent of his cases; and Alvarenga ('69) found practically no instances of obliteration of the foramen ovale or ductus arteriosus before sixty days. The findings of later observers (Alexeieff ('00), Theremin ('87–'95), Kucheff ('01) and others) agree essentially with those of Elsässer and Alvarenga although they have noted some instances of earlier obliteration of the passages.[2]

THE FORAMEN OVALE

The compiled data on the obliteration of the foramen ovale are shown in extenso in table 1. Table 2 is a summary of these data giving by periods the total number of observations and the number and per cent of cases obliterated. Graphic representations of these data are shown in figure 1 curve A, and in figure 2. In the summary and curve the data of Billard are omitted because his findings are so directly opposed to those of all other investigators that we conclude that either his method of investigation was defective or that his definition of obliteration was entirely different.

As will be seen in table 2, less than one per cent of the foramina are completely closed in the first week of life, and less than two and one-half per cent in the second week. In the latter part of the first month the figures indicate that the obliterative process takes place more rapidly as the opening is impervious in about one-eighth of all cases of this period. The rapidity of this process increases during the second month, for the interatrial

---

[1] The cases reported by Bernutz were not examined by him personally but were collected at his instance by the interne of a colleague in the Hospice des Enfants-Trouvés.

[2] Haberda ('96) studied the obliteration of the ductus venosus and ductus arteriosus in a considerable series of infants and children. As his data are not given in numerical form we are unable to include them in our summary. The general statements of this writer indicate that his findings were somewhat similar, as regards these two vessels, to our own.

TABLE 1

Data on the obliteration of the Foramen ovale. Numerals enclosed in parentheses indicate number of obliterated cases

OBSERVER AND DATE	0 TO 8 DAYS	8 TO 15 DAYS	15 TO 22 DAYS	22 TO 32 DAYS	32 TO 46 DAYS	46 TO 61 DAYS	61 TO 91 DAYS	3 TO 6 MONTHS	6 TO 9 MONTHS	9 TO 12 MONTHS	1 TO 5 YEARS	5 TO 10 YEARS	10 TO 20 YEARS	20 YEARS AND OVER
Alexeieff, '00.	3	7	6	11	21	25 (1)	46 (3)	68 (6)	15 (3)	3	2 (2)	1 (1)	1 (1)	
Alvarenga, '67	44	19	17	33	28	12	23 (2)	18 (1)	1	4	12 (4)		2 (1)	
Billard, '28...	118 (17)	20 (11)												
British Anat. Soc., '98...											23 (11)	9 (7)	30 (23)	286 (217)
Bizot, '37....											* 34 (23)			63 (49)
Elsässer, '52.	150 (1)	63 (4)	62 (9)	23 (2)	3 (1)									
Fawcett......													6 (3)	299 (207)
Letourneau, '58	8 (1)	1												
Ogle, '57.....														62 (49)
Theremin '87	70	76	63 (10)	49 (13)	55 (17)	45 (18)	81 (47)	65 (51)	11 (9)	7 (7)				
Theremin '95	18	5	4	6 (1)		4 (2)	8 (3)	6 (6)	† 6 (6)		4 (4)			
Wallmann, '59											2	2	4	291 (170)
Zahn.........														711 (552)
Totals.....	293 (2)	171 (4)	152 (19)	121 (16)	107 (18)	86 (21)	158 (55)	157 (64)	27 (12)	20 (11)	43 (21)	12 (8)	42 (28)	1712 (1228)

* 1 to 15 years

† 7 to 10 months.

TABLE 2

Obliteration of the Foramen ovale (3258 cases)

AGE	TOTAL NUMBER OF CASES	NUMBER OF CASES BLITERATED	PER CENT OF CASES OBLITERATED
Birth to 8 days	293	2	0.7
8 to 15 days	171	4	2.3
15 to 32 days	273	35	12.8
32 to 46 days	107	18	16.8
46 to 61 days	86	21	24.8
61 to 91 days	158	55	34.8
3 to 6 months	157	64	40.7
6 to 9 months	27	12	44.4
9 to 12 months	20	11	55.0
1 to 5 years	43	21	50.0
1 to 15 years	93	52	55.9
5 to 10 years	12	8	66.6
10 to 20 years	42	28	66.6
15 years and over	1840	1320	71.7
20 years and over	1712	1228	71.7

communication is obliterated in approximately one-sixth of all cases in the first half of this period and in about one-fourth in the latter half. During the third month somewhat less than ten per cent of the cases are obliterated so that by the end of the first trimester the foramen ovale is finally closed in about one-third of all cases. After the end of the third month the process again goes on more slowly and the average of obliteration in the second trimester is about forty per cent, that in the third trimester about forty-five per cent and that in the fourth trimester about fifty-five per cent. Our figures for the period between one and five years show an average obliteration of fifty-five per cent, which is five per cent less than that of the last trimester of the first year. This difference is due, no doubt, to the small number of cases tabulated in these two periods, and does not represent a real increase in the number of patent foramina. The figures of the second five year interval indicate that two-thirds of the foramina are closed; but the small amount of data for the period between the first and tenth years makes it impossible to say with certainty just when this increase in obliteration is brought about. In the second decade the percentage of open foramina is the same as in the period between five and ten years and the foramen ovale is found to be obliterated in about seventy-two per cent of individuals of twenty years and over.

Curve A of figure 1 expresses graphically the frequency of obliteration of the foramen ovale during different periods in the first year of life. This curve is readily divisible into three parts. The first portion, which extends from birth to the middle of the second week, is a short segment expressing the obliteration of a little over two per cent of the cases. It is followed by a longer segment rising abruptly and terminating in about the middle of the third month. Nearly half of the cases which are finally closed are obliterated in the period represented by this segment (sixty days). The third segment, extending from about the middle of the third month to the end of the year, shows a very slow but continuous rise and expresses the obliteration of about ten per cent of the total number of cases.

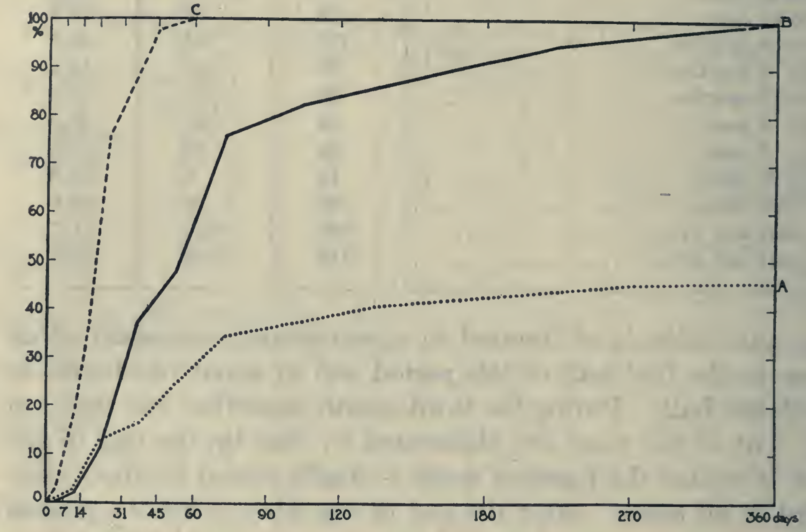


Fig. 1 Three curves representing the average percentages of obliterated fetal blood-passages at different periods in the first year of life. *A*, dotted line, foramen ovale, *B*, solid line, ductus arteriosus; *C*, broken line, ductus venosus. These curves are based upon the material summarized in tables 2, 4 and 5.

Figure 2 is a curve illustrating the frequency of the obliteration of the foramen ovale throughout life. The details of the obliteration during the first year, which have just been described, are masked in this figure by the diminution of the time unit. Here again three periods can be recognized which correspond roughly to infancy, childhood, and adolescence and maturity.

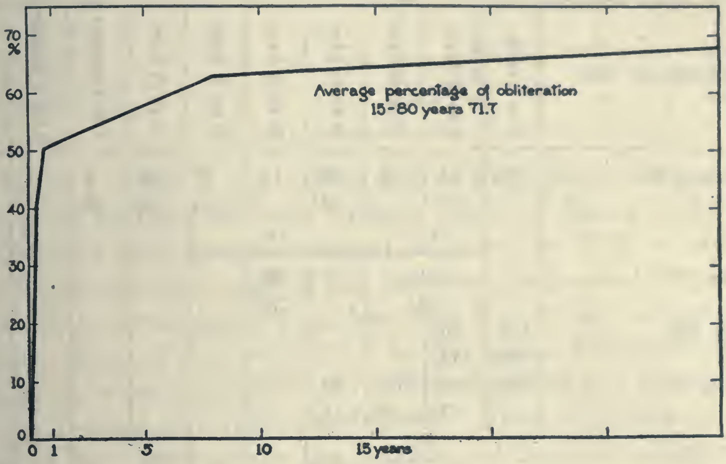


Fig. 2 A curve showing the average percentages of cases of obliterated foramen ovale at different periods throughout life. Based upon the material summarized in table 2.

During the first period the curve rises with extreme abruptness to a point at which fifty per cent of the cases are obliterated. The rise is continued but is much less rapid during the second period which extends from infancy well into childhood. In the third period, which extends to extreme old age, the curve rises very slowly to about seventy-two per cent. In all probability this final percentage of obliteration is reached in early maturity although the character of our data does not permit a graphic representation of this point.

THE DUCTUS ARTERIOSUS

Table 3 shows the compiled data upon the post-natal obliteration of the ductus arteriosus, and table 4 summarizes these data by periods. The graphic presentation of this material is shown

TABLE 3

Data on the frequency of post-natal obliteration of the Ductus arteriosus. Numerals enclosed in parentheses indicate number of obliterated cases

<table>
<tr><th>OBSERVER AND DATE</th><th>0 TO 8 DAYS</th><th>8 TO 15 DAYS</th><th>15 TO 22 DAYS</th><th>22 TO 32 DAYS</th><th>32 TO 46 DAYS</th><th>46 TO 61 DAYS</th><th>61 TO 91 DAYS</th><th>3 TO 4 MONTHS</th><th>4 TO 12 MONTHS</th><th>1 TO 10 YEARS</th></tr>
<tr><td>Alverenga, '69</td><td>19</td><td>11</td><td>15</td><td>28
(1)</td><td>17</td><td>8
(1)</td><td>14
(5)</td><td>9
(3)</td><td>1</td><td>7
(7)</td></tr>
<tr><td></td><td></td><td colspan="2">*</td><td colspan="3">**</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Bernutz, '65</td><td></td><td colspan="2">21
(14)</td><td colspan="3">38
(36)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Billard, '28</td><td>118
(16)</td><td>20
(11)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Elsässer, '52</td><td>150
(1)</td><td>63
(3)</td><td>61
(6)</td><td>23
(1)</td><td>3</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td colspan="4">°</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Faber, '12</td><td colspan="4">5</td><td>3</td><td></td><td colspan="2">15
(14)</td><td>6
(6)</td><td>23
(18)</td></tr>
<tr><td>Gérard, '00</td><td>52</td><td>2</td><td>2
(2)</td><td>4</td><td>2
(2)</td><td>2
(2)</td><td>2
(2)</td><td></td><td>16
(12)</td><td>13
(13)</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td colspan="2">†</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Kucheff, '01</td><td></td><td></td><td></td><td></td><td colspan="2">40
(18)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Letourneau, '58</td><td>12</td><td>1</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Theremin, '87</td><td>67</td><td>66</td><td>61
(6)</td><td>56
(8)</td><td>50
(26)</td><td>43
(23)</td><td>68
(55)</td><td>53
(48)</td><td>54
(54)</td><td></td></tr>
<tr><td>Theremin '95</td><td>11</td><td>5</td><td>4
(2)</td><td>6
(3)</td><td></td><td>4
(1)</td><td>8
(8)</td><td>1
(1)</td><td>12
(12)</td><td>4
(4)</td></tr>
<tr><td>Totals</td><td>429
(17)</td><td>168
(14)</td><td>143
(18)</td><td>117
(13)</td><td>75
(28)</td><td>57
(27)</td><td>92
(70)</td><td>63
(52)</td><td>89
(84)</td><td>47
(33)</td></tr>
</table>

* 10 to 20 days.

** 21 to 60 days.

† 2nd month (data for other periods not available).

° 0 to 30 days.

TABLE 4

Obliteration of the Ductus arteriosus (1095 cases)

AGE	TOTAL NUMBER OF CASES	NUMBER OF CASES OBLITERATED	PER CENT OF CASES OBLITERATED
Birth to 8 days	311	1	0.3
8 to 15 days	148	3	2.0
15 to 22 days	143	16	11.2
22 to 32 days	117	13	11.1
32 to 46 days	75	28	37.3
46 to 61 days	57	27	47.4
61 to 91 days	92	70	76.0
91 to 120 days	63	52	82.5
120 to 365 days	89	84	94.5

in figure 1, curve B. As in the case of the foramen ovale, and for the same reasons, we have omitted Billard's data and also those of Bernutz from the summarized table and from the curve.

During the first week of life the percentage of obliteration of the ductus arteriosus is even less than that of the foramen ovale (three-tenths of one per cent). In the second week the percentage rises to an average of two and in the third and fourth weeks to an average of a little over eleven. From this time on to the end of the third month the process of obliteration is extremely rapid; in the first part of the second month it averages over thirty-seven per cent, in the latter part over forty-seven, and in the third month seventy-six per cent. During the fourth month the average obliteration is eighty-two per cent. Thereafter the percentage increases quite slowly until the end of the year. The average percentage of obliteration in the last three-quarters of the first year is nearly ninety-five.

The data available for the period after one year are small in amount and, with the exception of certain instances in Faber's series, all cases of this period were obliterated. It is quite possible that Faber's material included several specimens which, while containing remnants of the original lumen, were obliterated at other points. His method of examination might easily classify such cases as patent. It is probable, therefore, that table 3 shows a much lower per cent of obliteration for this period than is actually the case.

Curve B of figure 1 is the graphic presentation of the data summarizes above. Like the curve for the foramen ovale already described and represented in the same figure, this graph is readily divisible into three portions. The first segment extends from birth to the middle of the second week and shows a terminal obliteration of two per cent of the cases. The second segment rises abruptly, crosses that of the foramen ovale, and terminated at about seventy-five per cent in the middle of the third month. The third segment, which extends from the middle of the third month to the end of the first year, shows a continuously decreasing rate of obliteration and terminates at nearly one hundred per cent. Probably all normal cases are closed shortly after the first year, although there are numerous records of individual cases of the anomalous persistence of the lumen of the ductus arteriosus in later life.[3]

THE DUCTUS VENOSUS

The material compiled upon the obliteration of the ductus venosus is shown in table 5 and is represented graphically in curve A of figure 1.

TABLE 5

Obliteration of the Ductus venosus (762 cases)

AGE	TOTAL NUMBER OF CASES	NUMBER OF CASES OBLITERATED	PER CENT OF CASES OBLITERATED
Birth to 8 days	211	6	2.3
8 to 15 days	150	27	18.0
15 to 22 days	169	64	37.5
22 to 32 days	103	78	75.7
Second month	37	36	97.3
Third month	20	20	100.0
Fourth, fifth, and sixth months	41	41	100.0
6 months to 1 year	19	19	100.0
1 year and over	12	12	100.0

---

[3] For the literature on the subject of persistent patency of the ductus arteriosus the reader is referred to the papers of Poynter ('16), Wells ('08) and Gerard ('00[2]).

The process of obliteration is much more rapid in the ductus venosus than in the other fetal passages. During the first week the average is two and three-tenths per cent, in the second week it is eighteen per cent, in the third thirty-seven and one-half per cent, and in the last ten days of the first month about seventy-six per cent. During the second month the percentage rises to nearly one hundred and thereafter all cases are obliterated.

The curve shown in figure 1, while much more abrupt than that of the foramen ovale and the ductus arteriosus, is of the same general character and shows three segments. The first segment corresponds to the first few days after birth, and terminates between two and three per cent. The second segment rises with extreme abruptness to ninety-seven per cent in the middle of the second month. The third segment, which is very short, rises gradually to a full hundred per cent by the end of this month.

THE RATE OF THE OBLITERATIVE PROCESS IN THE SEVERAL PASSAGES

In order to study the *activity* of the obliterative process in the various fetal blood-passages we have calculated from the graphs shown in figure 1 the average daily rate of obliteration for a series of periods in early life. This was done by determining from the graphs the initial and terminal percentages of obliteration for each given period. The initial percentage was then subtracted from the terminal one and the figure thus obtained divided by the number of days in the period. For example, in the case of the ductus arteriosus the percentage of obliteration at the beginning of the second month was fifty-seven and at the close was seventy-nine and one-half. Thus twenty-two and one-half per cent of all cases were obliterated in this period of thirty days and the average daily rate of obliteration was seventy-five hundredths per cent. Table 6 shows the results of these calculations for the three passages and the curves in figure 3 express them graphically.

It will be seen by the examination of these curves that they have certain characters in common. Each starts with a low rate

TABLE 6

Approximate rate of daily obliteration of the Ductus venosus, Ductus arteriosus, and Foramen ovale in early childhood

PERIOD	DUCTUS VENOSUS	DUCTUS ARTERIOSUS	FORAMEN OVALE
1st week	0.33	0.04	0.10
1 week to 1 month	3.00	0.92	0.60
2nd month	0.60	1.60	0.45
3rd month	0	0.75	0.40
3 to 6 months	0	0.07	0.07
6 to 12 months	0	0.04	0.04
1 to 5 years	0	0	0.005

of obliteration, rises rapidly to a peak or maximum, and then declines to the base-line which represents the cessation of obliterative activity. In all cases the portion of the curve representing the decline in activity is less abrupt than is the initial rise.

Considering the curves individually, it will be noted that in the case of the ductus venosus both the initial and maximal rate of obliteration is much greater than that of the other two passages and that consequently the entire obliterative process is completed much sooner. The curve expressing the rate of obliteration of the ductus arteriosus is very similar to that of the ductus venosus although the initial and maximal rates are lower and the apex of the curve falls at a later period. While the curve of the rate of obliteration of the foramen ovale shows the three common characters indicated in the preceding paragraph it is markedly different from the curves of the vessels. It rises less abruptly to a lower maximum rate of obliteration which is maintained with but little loss for a much longer period, so that the curve presents a plateau which is entirely absent from the curves of the vessels. Following this plateau, the curve falls at first rapidly and then gradually over a long interval to the base line.

The results of this study may be summarized as follows:

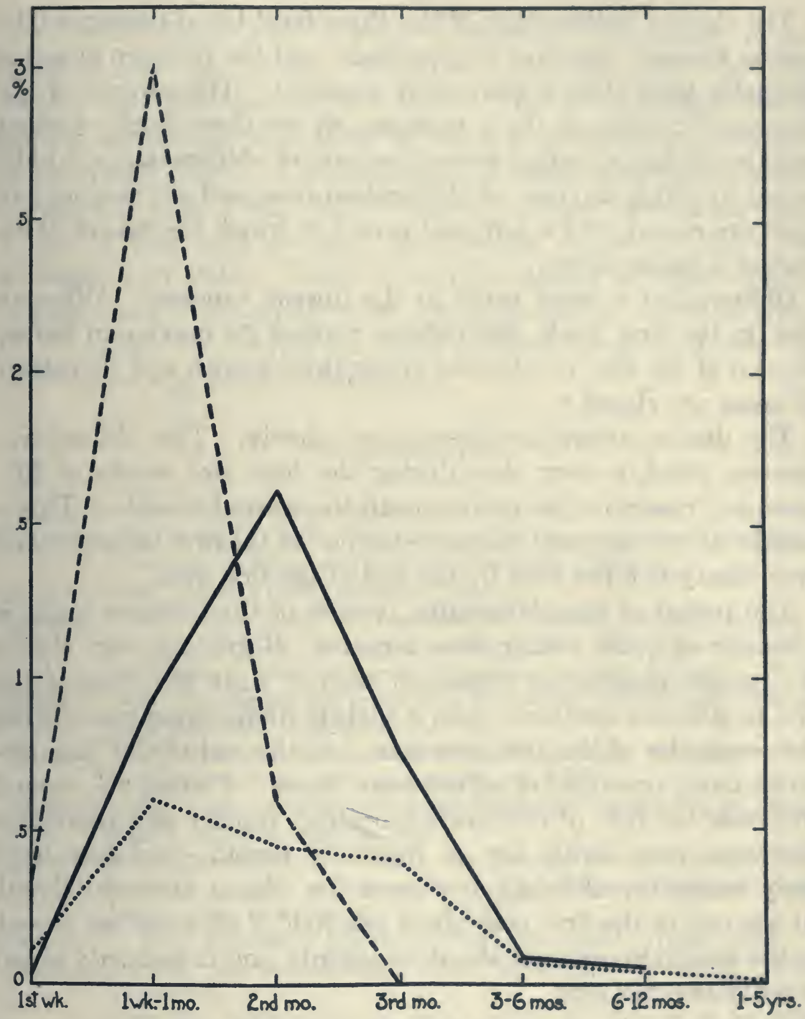


Fig. 3 Three curves showing the approximate average daily rate of obliteration of the fetal blood-passages in early life. *A*, dotted line, foramen ovale; *B*, solid line, ductus arteriosus; *C*, broken line, ductus venosus. Based upon the data summarized in table 6.

SUMMARY

The time of obliteration of the three fetal blood-passages (the ductus venosus, the ductus arteriosus, and the foramen ovale) is distinctly later than is commonly assumed. The process of obliteration in each of these passages shows three fairly distinct periods: an initial period with a low rate of obliteration, a middle period in which the rate of obliteration rises and the majority of cases are closed, and a terminal period in which the rate of obliteration is again slower.

Obliteration is most rapid in the ductus venosus. Although slow in the first week, the process reaches its maximum before the end of the first month and in the third month and thereafter all cases are closed.[4]

The ductus arteriosus closes more slowly. The obliterative process, which is very slow during the first two weeks of life, does not reach its maximum until the second month. Three-fourths of all cases are closed at the end of the first trimester and over ninety-five per cent by the end of the first year.

The period of the obliterative process of the foramen ovale is a matter of years rather than months. Beginning very slowly the process reaches its maximum activity near the close of the first month and continues with a slightly diminishing rate during the remainder of the first trimester. At the end of this time approximately one-third of all cases are closed. During the second trimester the rate of obliteration declines rapidly and thereafter decreases very slowly for an indefinite period—probably until early maturity, although few cases are closed after childhood. At the end of the first year about one-half of all cases are closed, in the second decennium about two-thirds, and in maturity about seventy-two per cent.

---

[4] It has been shown by the studies of Wertheimer ('86), Nikitin ('01), Fontan ('11) and others that the vein which sometimes occupies the center of the ligament of the ductus venosus in older children and adults is a new vessel developed after the obliteration of the ductus venosus and is not derived from the remains of this trunk.

POSTSCRIPT

After this paper was in press we secured a summary of one hundred and eighty-seven observations by Parrot on the obliteration of the ductus arteriosus. Parrot's findings are in general agreement with those of other observers which we have summarized above. Unfortunately his cases under one year are grouped in such a way that we cannot include them in our table 4, but if this were possible they would evidently affect our averages very little. Parrot found the ductus arteriosus patent in four cases in thirty-three of one year and in one in fifty-four of two years. In seventeen cases of three years and over the the ductus was always obliterated.

BIBLIOGRAPHY

ALEXEIEFF 1900 The foramen ovale in the child. Diss. St. Petersburg.

ALVARENGA, P. F. DaC. 1869 Considérations et observations sur l'époque de l'occlusion du trou ovale et du canal artériel. Lisbon.

BERNUTZ 1865 [Quoted from Gérard, G. ('00²).]

BILLARD, C. M. 1828 Traité des maladies des enfants nouveau-nés et à la mamelle, fondé sur de nouvelles observations cliniques et d'anatomie pathologique, faites à l'hopital des enfants trouvés de Paris. Paris.

BIZOT, J. 1837 Recherches sur le coeur et le système artériel chez l'homme. Mem. Soc. Med. d'Observation, T. I.

ELSÄSSER 1852 Über den Zustand der Fötuskreislaufwege bei neugeborenen Kindern. Zeitschr. f. Staatsarzneik., Bd. LXIV.

FABER, A. 1912 Die anatomischen und physikalischen Verhältnisse des Ductus Botalli. Arch. f. Anat. u. Ent.

FAWCETT E. AND BLACHFORD, J. V. 1900 The frequency of an opening between the right and left auricles at the seat of the foetal foramen ovale. Journ. Anat. and Physiol., vol. XXXV.

FONTAN, C. 1911 Le canal d'Arantius (étude anatomique). Thèse, Lille.

GÉRARD, G. 1900 Le canal artériel. Étude anatomique. Journ. de l'Anat., T. XXXVI.

1900² De l'oblitération du canal artériel (les théories et les faits). Journ. de l'Anat., T. XXXVI.

HABERDA, A. 1896 Die fötalen Kreislaufswege des Neugeborenen und ihre Veränderungen nach der Geburt. Wien.

KLOBB 1859 (Amtl. Ber. XXXIII Versamml. deutsch. Naturf. u. Arzte zu Bonn, 1857.)

---

[5] Depaul, Dict. Encyclopéd. d. Sc. Méd., 2. sér., T. XIII.

KUCHEFF, N. E. 1901 (The ductus Botalli in children.) Diss. St. Petersburg (Quoted from summaries in Jahresber. f. Anat. u. Entwickl., Bd. VII (N.F.), and from Gundobin: Die Besonderheiten des Kindesalters, 1912.)

LETOURNEAU 1858 Quelques observations sur le nouveau-né. Thèse, Paris.

PARSONS, F. G. AND KEITH, A. 1898 Seventh report of the committee of collective investigation of the Anatomical Society of Great Britain and Ireland, for the year 1896–97. Journ. of Anat. and Physiol., vol. XXXII.

NIKITIN, A. A. 1901 The duct of Arantius in childhood. Diss. St. Petersburg.

OGLE, J. 1857 On certain cases in which the foramen ovale was still patent in the adult. British Med. Journ.

POYNTER, C. W. M. 1916 Arterial anomalies pertaining to the aortic arches and the branches arising from them. University Studies, (U. of Nebraska) vol. XVI.

QUINCKE, H. 1885 Ueber die Entstehung der Gelbsucht Neugeborener. Arch. f. exp. Pathol. u. Pharmakol., Bd. XIX.

THÉREMIN, E. 1887 Note sur l'involution des voies foetales. Rev. d. Mal. d. l'Enfance, T. V.

1895 Études sur les affections congenitales du coeur. Paris.

WALLMANN, H. 1859 Ueber das Offenbleiben des Foramen ovale cordis bei Erwachsenen. Vierteljahrschr. f. d. prakt. Heilk., Bd. LXII.

WELLS, H. G. 1908 Persistent patency of the ductus arteriosus. Amer. Journ. Med. Sc., N.S., vol. CXXXVI.

WERTHEIMER, E. 1886 Recherches sur la veine ombilcale. Journ. de l'Anat., T. XXII.

ZAHN, (Cited by Abbot, M. E. in: Osler and McCrae, Modern Medicine, vol. IV, 1908.)

Ho. 18. 301

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 2

Reprinted from THE JOURNAL OF EXPERIMENTAL
ZOÖLOGY, Vol. 25, No. 2, April, 1918

CHANGES IN THE RELATIVE WEIGHTS OF THE VARIOUS PARTS, SYSTEMS AND ORGANS OF YOUNG ALBINO RATS UNDERFED FOR VARIOUS PERIODS

CHESTER A. STEWART

Institute of Anatomy, University of Minnesota, Minneapolis

ONE FIGURE AND FOUR TABLES

CONTENTS

Material and methods 302
Ratio of tail length to body length 318
Head 319
Extremities and trunk 320
Integument 321
Skeleton 322
Musculature 326
Viscera 327
'Remainder' 327
Brain 328
Spinal cord 330
Eyeballs 330
Thyroid gland 332
Thymus 333
Heart 334
Lungs 335
Liver 336
Spleen 337
Stomach and intestines 338
Suprarenal glands 339
Kidneys 340
Testes and epididymi 341
Ovaries 342
Hypophysis 343
Pineal body 344
Discussion 345
Summary 347
Literature cited 352

It has recently been shown that although growth in body weight in the young rat may be completely suppressed by underfeeding for considerable periods, nevertheless, certain organs and parts of the body having a very strong growth tendency continue to increase in weight, other organs and parts with a weaker growth capacity remain nearly unchanged, while still other parts suffer a loss in weight (Jackson '15 b and Stewart '16). These changes were observed chiefly in rats whose underfeeding began at the age of three weeks and terminated at the age of ten or twelve weeks. Very few observations have been recorded, however, concerning such changes which may occur either in younger animals subjected to inanition for various periods, or in animals underfed for much longer periods. An investigation was therefore undertaken in order to determine these changes. The work was done in the Institute of Anatomy of the University of Minnesota, under the supervision of Dr. C. M. Jackson, to whom I am indebted for valuable aid and direction.

MATERIAL AND METHODS

For the present investigation eighty-nine albino rats (Mus norvegicus albinus) were used (table 1), all of which were autopsied at the close of the experiment. They included thirty-four controls and fifty-five test rats.

From the litters used, the controls were selected at the beginning of the experiment, the sex being determined according to the method of Jackson ('12). Seven (4 M, 3 F) of the controls were killed and autopsied when they had reached (on full feeding) an approximate average net body weight of 10 grams, four ([illegible] M, 1 F) at 13 grams, fourteen (7 M, 7 F) at 15 grams, one (M) at 27 grams, one (F) at 40 grams, four (2 M, 2 F) at 50 grams, and three (2 M, 1 F) at 70 grams. Whenever possible, the controls were selected from the same litters as the test rats. In some instances, however, this was not possible, especially in the case of the controls for the rats underfed for very long periods.

The majority of the test rats were starved for intermittent periods starting a short time (24 to 48 hours) after birth, and were killed (by chloroform) and autopsied at the age of three weeks (17 rats; 9 M, 8 F), six weeks (9 rats; 6 M, 3 F), and ten weeks

TABLE 1

Average and range of gross and net body weight, body length, tail ratio, and weights of the head, trunk, extremities, skeleton, musculature visceral group and 'remainder'

Test rats in first six groups were underfed from birth; last six groups from age of three weeks

DESCRIPTION OF RATS, NUMBER, SEX AND AGE	GROSS BODY WEIGHT	NET BODY WEIGHT	BODY LENGTH	RATIO OF TAIL LENGTH TO BODY LENGTH
	grams	*grams*	*mm.*	
4 Control M 7.5(7–8)da.	10.6(10.0–11.2)	9.82(9.3–10.3)	65.5(64.0–67.0)	46.9(40.6–56.7)
9 Test M 21.2(20–23)da.	10.2(9.5–11.5)	9.90(9.1–11.2)	68.0(66.0–69.0)	59.0(57.4–63.6)
3 Control F 7 da.	10.5(10.0–10.8)	9.77(9.3–10.1)	64.7(62.0–66.0)	45.4(43.9–47.0)
8 Test F 22.2(21–28)da.	10.5(9.5–11.5)	10.20(9.1–10.9)	69.8(66.0–76.0)	66.3(52.9–71.2)
3 Control M 14(13–17)da.	13.6(13.5–13.6)	12.97(12.6–13.3)	71.0(70.0–72.0)	57.2(56.3–58.3)
6 Test M 47(42–59)da.	14.1(13.1–15.9)	12.50(11.3–13.3)	82.3(77.0–87.0)	68.2(67.1–69.0)
1 Control F 13 da.	13.2	12.76	71.0	60.6
3 Test F 42 da.	13.8(13.0–14.9)	12.82(12.2–13.7)	78.3(76.0–80.0)	75.3(74.7–76.3)
7 Control M 12.6(11–15)da.	15.9(15.0–16.9)	14.95(14.2–15.9)	75.2(71.0–79.0)	53.7(47.9–56.6)
6 Test M 69.2(64–72)da.	15.5(14.4–17.0)	14.29(13.4–15.6)	86.8(83.0–89.0)	73.9(62.4–85.5)
7 Control F 12.9(11–15)da.	15.6(15.0–16.2)	14.71(13.8–15.7)	75.3(74.0–77.0)	56.6(53.9–60.8)
7 Test F 69.1(67–72)da.	16.1(14.0–17.1)	14.86(13.2–15.7)	84.9(81.0–88.0)	82.0(76.1–87.7)
1 Control M 22 da.	29.6	27.25	98.0	73.5
1 Test M 139 da.	30.0	28.67	118.0	89.0
1 Control F 37 da.	44.5	39.99	116.0	78.4
1 Test F 316 da.	42.4	39.63	130.0	
2 Control M 36(35–37)da.	51.6(50.5–52.8)	49.10(47.5–50.7)	120.5(120.0–121.0)	65.5(65.2–65.8)
4 Test M 412(374–428)da.	48.6(44.1–52.4)	46.02(40.9–49.9)	134.3(132.0–140.0)	88.3(84.1–93.2)
2 Control F 44(43–45)da.	53.3(50.0–56.5)	48.55(45.2–51.9)	122.5(121.0–124.0)	83.7(83.5–83.9)
4 Test F 392(377–428)da.	54.1(49.0–56.5)	50.41(45.3–53.9)	138.5(135.0–148.0)	93.1(89.2–97.1)
2 Control M 47(46–48)da.	76.5(76.0–77.0)	71.93(70.5–73.4)	140.5(140.0–141.0)	78.95(72.1–85.8)
4 Test M 291(223–316)da.	77.6(66.8–101.5)	69.87(63.4–83.2)	152.8(150.0–157.0)	92.70(87.9–94.8)
1 Control F 53 da.	73.0	67.85	137.0	79.60
2 Test F 314(311–316)da.	81.6(67.0–96.2)	67.20(58.1–76.3)	149.0(146.0–152.0)	92.25(91.1–93.4)

TABLE 1—Continued

HEAD	UPPER EXTREMITIES	LOWER EXTREMITIES	TRUNK	INTEGUMENT
grams	*grams*	*grams*	*grams*	*grams*
2.35(2.29–2.50)	0.686(0.454–0.814)	0.930(0.684–1.090)	5.856(5.305–6.558)	1.999(1.800–2.109)
2.78(2.60–2.90)	0.797(0.734–0.912)	1.191(1.089–1.294)	5.130(4.492–6.321)	2.095(1.553–2.760)
2.51(2.44–2.60)	0.668(0.494–0.806)	0.940(0.650–1.101)	5.648(4.892–6.548)	2.124(1.940–2.279)
2.88(2.70–3.00)	0.804(0.697–0.901)	1.237(1.036–1.371)	5.285(4.630–5.662)	2.032(1.407–2.600)
3.40(3.30–3.50)	1.168(1.120–1.204)	1.596(1.565–1.657)	6.839(6.564–7.114)	3.181(2.920–3.450)
3.30(3.10–3.50)	0.928(0.855–0.992)	1.649(1.588–1.767)	6.635(5.625–7.437)	1.921(1.714–2.116)
3.40	1.176	1.606	6.578	3.340
3.23(2.90–3.50)	0.918(0.891–0.959)	1.626(1.465–1.751)	7.041(6.657–7.513)	1.784(1.578–1.925)
3.70(3.50–3.90)	1.208(1.000–1.306)	1.842(1.611–2.000)	8.209(7.644–8.945)	3.895(3.540–4.700)
3.80(3.50–4.10)	1.045(0.917–1.137)	1.899(1.690–2.012)	7.564(6.834–8.505)	2.064(1.650–2.318)
3.70(3.40–3.80)	1.194(1.000–1.325)	1.869(1.710–2.000)	7.965(7.150–8.722)	3.909(3.414–4.500)
3.90(3.80–4.10)	1.079(0.912–1.262)	1.970(1.771–2.143)	7.913(6.417–8.712)	2.018(1.872–2.534)
5.10	2.210	4.167	15.771	4.730
6.00	2.100	4.600	15.966	4.400
6.40	3.105	6.900	23.585	7.118
8.00	2.992	6.095	22.547	6.515
6.90(6.7–7.1)	3.611(3.600–3.622)	7.534(7.369–7.700)	31.054(29.809–32.300)	9.413(9.400–9.426)
8.70(7.4–9.3)	3.477(2.587–3.911)	6.998(5.576–7.889)	26.842(25.357–28.843)	7.449(5.361–8.900)
6.85(6.4–7.3)	3.487(3.280–3.693)	7.697(7.240–8.154)	30.496(28.253–32.739)	9.231(8.820–9.641)
8.75(8.4–9.2)	3.816(3.400–4.199)	8.180(6.823–9.502)	29.662(26.386–34.161)	9.174(8.164–10.000)
8.75(8.7–8.8)	4.964(4.798–5.130)	11.577(11.524–11.630)	46.665(45.478–47.852)	14.055(12.334–15.777)
10.55(9.8–11.2)	6.033(5.200–6.900)[1]	14.200(12.800–15.200)[1]	39.400(32.100–50.500)[1]	17.300(11.268–22.200)[1]
8.60	5.096	11.757	42.392	12.713
10.35(10.1–10.6)	5.550(4.800–6.300)	13.700(11.600–15.800)	37.600(31.600–43.600)	18.700)[4]

TABLE 1—Continued

LIGAMENTOUS SKELETON	MOIST CARTILAGINOUS SKELETON	DRY CARTILAGINOUS SKELETON	MUSCULATURE	VISCERAL GROUP	REMAINDER
grams	*grams*	*grams*	*grams*	*grams*	*grams*
1.636(1.393–1.799)	0.715(0.481–0.959)[1]	0.145(0.118–0.184)[1]	2.244(1.590–2.473)	1.977(1.891–2.153)	1.964(1.664–2.686)
1.915(1.767–2.092)	1.610(1.398–1.887)	0.359(0.290–0.451)[2]	2.442(2.197–2.986)	2.501(2.271–2.719)	1.076(0.534–2.027)[8]
1.598(1.509–1.700)	0.856(0.543–1.168)[3]	0.136[4]	2.356(2.020–2.745)	2.002(1.896–2.135)	1.683(1.371–2.137)
1.923(1.610–2.256)	1.440(1.216–1.897)	0.389(0.328–0.492)[2]	2.530(1.640–2.890)	2.606(2.322–2.982)	1.109(0.583–1.554)
2.302(2.225–2.394)	1.699(1.519–1.865)	0.329(0.289–0.359)	3.053(2.953–3.119)	2.729(2.568–2.956)	1.705(1.184–2.129)
2.686(2.536–2.867)	2.331(1.891–2.485)	0.554(0.483–0.753)	3.338(2.979–3.608)	3.512(3.184–3.905)	1.104(0.000–2.038)
2.297	1.815	0.342	3.190	2.787	1.146
2.599(2.386–2.715)	2.350(2.308–2.418)	0.526(0.422–0.630)	3.659(3.510–3.860)	3.563(3.400–3.745)	1.213(0.622–1.695)
2.372(2.130–2.714)	1.667(1.399–2.088)[6]	0.376(0.336–0.423)[1]	3.840(3.609–4.060)[5]	2.935(2.775–3.042)	1.877(1.686–2.131)[5]
2.937(2.796–3.153)	2.568(2.121–2.737)	0.767(0.662–0.856)[5]	3.966(3.368–4.467)	3.840(3.425–4.209)	1.486(1.028–2.088)
2.341(2.204–2.561)[5]	1.528(1.294–2.006)[7]	0.372(0.350–0.390)[7]	3.863(3.488–5.126)[5]	2.935(2.758–3.168)	1.694(0.105–2.564)[5]
2.894(2.700–3.013)	2.386(1.948–2.847)	0.731(0.683–0.856)	4.475(3.660–5.227)	4.204(3.768–5.065)	1.271(0.855–1.703)
4.305	3.587		8.175	5.453	4.585
5.400	4.300	2.079	8.500	5.388	4.978
5.844			14.448	7.448	5.132
8.838	7.957	3.081	12.393	7.668	4.220
6.785(6.620–6.951)	5.572(5.411–5.733)	1.603(1.336–1.870)	16.650(16.320–16.980)	8.605(8.298–8.912)	7.651(5.900–9.402)
8.151(7.558–8.796)	7.209(6.403–8.341)	3.523(2.897–4.035)	16.377(11.472–18.821)	8.893(7.934–11.283)	5.146(4.074–5.734)
6.207(6.066–6.347)	5.193[4]		16.447(15.684–17.209)	9.646(8.935–10.356)	7.000(5.668–8.333)
8.963(7.930–10.111)	7.774(7.055–8.813)[1]	3.942(3.340–4.415)	18.110(13.355–21.076)	9.537(7.026–13.744)	4.626(3.466–5.894)
8.457(7.903–9.011)	7.197(6.281–8.104)	2.503(2.229–2.776)	28.082(26.474–29.690)	13.680(13.025–14.336)	7.662(5.129–10.196)
12.500(11.300–13.800)[1]	9.030(8.800–9.300)[1]	4.273(3.738–4.890)	33.700(30.000–37.400)[3]	11.186(10.733–11.639)[3]	6.300[4]
8.202	7.232		26.221	10.254	10.455
11.550(11.100–12.000)	9.400(9.100–9.700)	3.856(3.692–4.020)	35.300[4]	10.698[4]	0.000

---

[1] Average of 3 individuals.
[2] Average of 7 individuals.
[3] Average of 2 individuals.
[4] Average of 1 individual.
[5] Average of 6 individuals.
[6] Average of 5 individuals.
[7] Average of 4 individuals.
[8] Average of 8 individuals.

(13 rats; 6 M, 7 F), the average net body weight at each age being approximately 10, 13 and 15 grams respectively.

In addition sixteen rats, starting at three weeks of age, were underfed for much longer periods (to 139 to 412 days of age; see table 1). They were then killed and autopsied. For one litter of the group fasting for a long period (M 29), the underfeeding started at the age of five weeks. Four rats of litters S 26 and M 29 (one male and one female of each litter), and one male of litter St 44 were accidentally asphyxiated by illuminating gas. These rats are all included in the two groups of test rats weighing approximately 70 grams. The two asphyxiated individuals of litter M 29 had been refed about thirty-six hours, which has resulted chiefly in a great increase in the contents of the alimentary canal.

In general the plan was to kill the test rats at the same body weight as the corresponding controls, but this was not always possible. Nevertheless, the average body weight of the different groups of test rats differs only slightly from that for the corresponding controls, as is shown in table 1. In comparing the data from the test rats with those from the controls throughout the paper, this difference has generally been disregarded, although strictly speaking, there should be a slight correction in every case for differences in body weight. Such a correction should be based upon the net body weight (excluding content of stomach and intestines), rather than upon the gross body weight, however. As the differences in body weight are in all cases small, it seems justified to ignore them in making comparisons.

The control rats remained constantly with the mother throughout the nursing period (three weeks). After this time they were fed an abundant diet of whole wheat (Graham) bread soaked in whole milk. Water was also supplied.

The test rats (in the experiments upon very young animals) were removed from the mother at frequent intervals for periods of usually 15 to 24 hours (occasionally longer, the maximum being 43 hours), and were permitted to nurse during the intervening time. For convenience, these test rats are frequently referred to throughout this paper as the rats fasting from birth for various periods. The total number of hours that the test rats of a litter were separated from the mother during the first three weeks (504 hours) after birth averaged 260 hours (213 to 285 hours) or more than half of the total period. Brüning ('14) was able to isolate young rats from the mother for only about one-fourth of the total nursing period.

In most instances when young fasting rats were returned to the nest, the mother would immediately take care of them. Occasionally, however, after being disturbed, the mother would abandon her litter entirely. In these cases it was often possible to save the young rats by putting them to nurse with other mothers whose young were fasting at that time. By continually putting young rats of different litters in the nest, it was found possible to keep one female nursing for twelve weeks.

The age of weaning for the albino rat is usually given as three weeks. However, Donaldson ('15) ('The Rat,' p. 19) states that the young, if permitted, will continue to depend partly on the mother for some days longer. One of my litters was observed to continue nursing until six weeks of age.

When three weeks old, the test rats underfed from birth, and also those used in the experiments starting at three weeks, were placed on the bread and milk diet, receiving only a limited and carefully measured quantity daily. As was observed by Jackson ('15 b) and Stewart ('16), the young rats in the present experiments were kept at maintenance for a considerable period upon a gradually diminishing ration. Thus for one litter (St 96) during 30 days underfeeding the daily amount of food required for maintenance of body weight at approximately 14.5 grams decreased from an average of 3.87 grams to 2.97 grams per rat. Later, however, the maintenance ration apparently becomes more nearly constant. For example, in the case of another litter (St 46) underfed for a very long period the total daily weight of food consumed by 5 rats from the 131st to the 180th day of the experiment was constantly 33 grams. The average body weight of these rats during this period remained unchanged (47.6 grams.) Stewart ('16) for one litter found no decrease in the maintenance ration from the 60th to the 120th day of the experiment.

The decrease in the maintenance ration noted in the young stunted rats is perhaps associated with the decrease in the intensity of metabolism which normally occurs with advancing age. Jackson ('15 b) suggests that during maintenance in young animals the amount of living protoplasm may be actually reduced, being replaced by water absorption, or that the food-intake may be more economically utilized under these conditions. He also mentions the decrease in body temperature as a possible factor. This problem is apparently of sufficient interest and importance to warrant a more exact and thorough investigation from the physiological point of view.

Since it was found very difficult to hold the test rats strictly at constant body weight and keep them alive for very long periods, a slight increase in body weight was usually permitted. Aron ('11), Jackson ('15 b) and Stewart ('16) similarly found it increasingly difficult to hold animals at constant body weight as the experiment progressed.

Separate weight records were kept for each rat, the individuals being identified by staining the integument with an alcoholic solution of picric acid. The identification marks had to be renewed morning and evening on the very young rats, but after the appearance of hairs the stains were very permanent. The young test rats were weighed daily immediately before feeding, whereas the controls, and also the test rats underfed for very long periods, were weighed at gradually increasing intervals as they grew older (about once in two weeks when about three months of age).

The cages used, and also the warm room in which the test rats were kept while fasting, have been described by Stewart ('16).

The autopsy technique employed by Jackson and Lowrey ('12) and Jackson ('13) was used with but few modifications. The various organs and parts were placed in a moist chamber when removed from the animal, and were weighed in a closed container on balances accurate to one-tenth milligram (0.0001 gram).

The data collected for the controls in this work were carefully compared with the published records of Jackson and Lowrey ('12), Jackson ('13 and '15), Hatai ('13 and '14), King ('15), and with the Wistar norm tables of Donaldson ('15). By this means it was possible to detect any marked variations from the normal in my control rats. For purposes of convenient comparison, the weights of organs from the Wistar tables for normal rats of body length corresponding to my controls have been inserted in table 2. In general, it will be noted that both the body weight and the corresponding organ weights are slightly higher in my controls than in the Wistar tables. If the comparison were based upon body weight rather than body length, there would usually be much closer agreement. While body length is a satisfactory basis for comparison between my controls and the Wistar data, it cannot be used in the case of the test rats, for as will appear later, the body length in underfed rats continues to increase even when the body weight is held constant. Body weight therefore was selected as a basis for comparison between my controls and test rats, since it can easily be held constant, which is not true for the body length.

In the present paper, for the most part only average data are published. However, a copy of the original individual observations will be deposited in The Wistar Institute of Anatomy and Biology, Philadelphia, where they may be consulted by those interested.

The normal variability of the various organs makes it necessary in some cases to exercise considerable care in drawing conclusions from the relatively limited number of observations. Nevertheless, the data appear sufficient to establish fairly accurately some of the more obvious and important changes due to the experiment.

An abstract of that part of the present investigation dealing with the underfeeding of the rats from birth to three, six and ten weeks of age was published in the Proceedings of the American Association of Anatomists, New York meeting, December, 1916 (Stewart, '17).

TABLE 2

Average (and range) of weight of the organs, with data from the Wistar tables (Donaldson, '15) for rats of body length corresponding to my controls. (In case of the thymus age is the basis of comparison)

Test rats in the first six groups were underfed from birth; last six groups from age of three weeks

DESCRIPTION OF RATS, NUMBER, SEX AND AGE	BODY LENGTH	GROSS BODY WEIGHT	NET BODY WEIGHT	BRAIN	SPINAL CORD
	mm.	*grams*	*grams*	*grams*	*grams*
4 Control M 7.5(7–8)da.	65.5(64.0–67.0)	10.6(10.0–11.2)	9.82(9.28–10.33)	0.609(0.577–0.634)	0.081(0.076–0.087)
Wistar data	65.0	9.4		0.650	0.071
9 Test M 21(20–23)da.	68.0(66.0–69.0)	10.2(9.5–11.5)	9.90(9.12–11.20)	0.975(0.839–1.034)	0.137(0.114–0.157)
3 Control F 7 da.	64.7(62.0–66.0)	10.5(10.0–10.8)	9.77(9.26–10.13)	0.625(0.608–0.644)	0.079(0.077–0.082)
Wistar data	65.0	9.9		0.679	0.077
8 Test F 22(21–28)da.	69.8(66.0–76.0)	10.5(9.5–11.5)	10.20(9.13–10.88)	0.996(0.934–1.035)	0.135(0.105–0.164)
3 Control M 14(13–17)da.	71.0(70.0–72.0)	13.6(13.5–13.6)	12.97(12.61–13.28)	0.962(0.907–1.025)	0.124(0.109–0.136)
Wistar data	71.0	11.8		0.840	0.091
6 Test M 47(42–59)da.	82.3(77.0–87.0)	14.2(13.1–15.9)	12.50(11.34–13.27)	1.045(0.972–1.088)	0.176(0.153–0.186)
1 Control F 13 da.	71.0	13.2	12.76	1.035	0.145
Wistar data	71.0	12.5		0.876	0.098
3 Test F 42 da.	78.3(76.0–80.0)	13.8(13.0–14.9)	12.82(12.21–13.72)	1.005(0.923–1.059)	0.184(0.160–0.196)
7 Control M 13(11–15)da.	75.2(73.0–79.0)	15.9(15.0–16.9)	14.95(14.24–15.95)	1.021(0.963–1.087)	0.119(0.100–0.129)
Wistar data	75.0	13.6		0.952	0.104
6 Test M 69(64–72)da.	86.8(83.0–89.0)	15.5(14.4–17.0)	14.29(13.45–15.57)	1.073(0.961–1.145)	0.199(0.174–0.211)

TABLE 2—Continued

7 Control F 13(11–15)da.	75.3(74.0–76.0)	15.6(15.0–16.2)	14.71(13.84–15.73)	1.016(0.950–1.086)	0.121(0.103–0.133)
Wistar data	75.0	14.3		0.974	0.112
7 Test F 69(67–72)da.	84.9(81.0–88.0)	16.1(14.0–17.1)	14.86(13.20–15.67)	1.117(1.084–1.180)	0.207(0.188–0.225)
1 Control M 22 da.	98.0	29.6	27.25	1.357	0.198
Wistar data	98.0	26.2		1.271	0.182
1 Test M 139 da.	118.0	30.0	28.67	1.418	0.285
1 Control F 37 da.	116.0	44.5	39.99	1.444	0.262
Wistar data	116.0	42.2		1.411	0.261
1 Test F 316 da.	130.0	42.4	39.63	1.636	0.409
2 Control M 36(35–37)da.	120.5(120.0–121.0)	51.6(50.5–52.8)	49.10(47.5–50.7)	1.554(1.521–1.588)	0.300(0.296–0.303)
Wistar data	121.0	44.4		1.448	0.265
4 Test M 412(374–428)da.	134.3(132.0–140.0)	48.6(44.1–52.4)	46.02(40.9–49.9)	1.570(1.496–1.664)	0.407(0.394–0.424)
2 Control F 44 (43–45)da.	122.5(121.0–124.0)	53.3(50.0–56.5)	48.55(45.2–51.9)	1.427(1.350–1.505)	0.282(0.276–0.287)
Wistar data	123.0	49.1		1.45	0.287
4 Test F 392(377–428)da.	138.5(135.0–148.0)	54.1(49.0–56.5)	50.41(45.3–53.9)	1.533(1.516–1.549)	0.408(0.372–0.468)
2 Control M 47(46–48)da.	140.5(140.0–141.0)	76.5(76.0–77.0)	71.95(70.5–73.4)	1.500(1.429–1.571)	0.322(0.294–0.351)
Wistar data	141.0	66.7		1.569	0.338
4 Test M 291(223–316)da.	152.8(150.0–157.0)	77.6(66.8–101.5)	69.79(63.4–83.2)	1.576(1.502–1.626)	0.415(0.363–0.466)
1 Control F 53 da.	137.0	73.0	67.85	1.487	0.312
Wistar data	137.0	65.5		1.540	0.341
2 Test F 314(311–316)da	149.0(146.0–152.0)	81.6(67.0–96.2)	67.20(58.1–76.3)	1.540(1.454–1.626)	0.363[5]

TABLE 2—Continued

EYEBALLS	THYROID	THYMUS	HEART	LUNGS	LIVER
grams	*grams*	*grams*	*grams*	*grams*	*grams*
0.065(0.058–0.070)	0.0030(0.0026–0.0034)	0.023(0.018–0.027)	0.074(0.064–0.086)	0.201(0.172–0.225)	0.37(0.33–0.42)
0.058	0.0026	0.020	0.066	0.127	0.45
0.090(0.081–0.097)	0.0030(0.0020–0.0036)	0.015(0.0069–0.027)	0.066(0.058–0.080)	0.147(0.129–0.163)	0.40(0.36–0.44)
0.064(0.062–0.067)	0.0027(0.0026–0.0029)	0.023(0.020–0.025)	0.073(0.063–0.084)	0.191(0.178–0.205)	0.38(0.35–0.41)
0.061	0.0028	0.020	0.069	0.131	0.48
0.094(0.083–0.098)	0.0030(0.0017–0.0053)	0.017(0.011–0.029)	0.074(0.063–0.090)	0.141(0.126–0.171)	0.48(0.37–0.63)
0.087(0.084–0.091)	0.0042(0.0032–0.0054)	0.037(0.031–0.040)	0.081(0.073–0.090)	0.210(0.197–0.235)	0.45(0.37–0.53)
0.069	0.0033	0.031	0.083	0.148	0.71
0.118(0.106–0.127)	0.0041(0.0018–0.0050)[1]	0.012(0.0050–0.020)	0.092(0.082–0.112)	0.149(0.126–0.169)	0.71(0.53–0.96)
0.092	0.0046	0.037	0.084	0.195	0.40
0.072	0.0034	0.029	0.087	0.154	0.79
0.118(0.110–0.124)	0.0047(0.0035–0.0060)	0.018(0.012–0.025)	0.095(0.090–0.104)	0.144(0.132–0.162)	0.83(0.78–0.89)
0.093(0.088–0.097)	0.0040(0.0028–0.0056)	0.048(0.049–0.056)	0.094(0.085–0.110)	0.230(0.207–0.272)	0.52(0.47–0.58)
0.076	0.0037	0.029	0.095	0.163	0.91
0.149(0.131–0.157)	0.0038(0.0031–0.0049)	0.0082(0.0051–0.0150)	0.109(0.094–0.131)	0.183(0.141–0.293)	0.82(0.69–1.00)
0.093(0.087–0.098)	0.0040(0.0032–0.0052)	0.048(0.041–0.057)	0.089(0.083–0.098)	0.238(0.216–0.263)	0.56(0.50–0.62)
0.078	0.0039	0.029	0.099	0.169	0.98
0.160(0.158–0.161)[2]	0.0045(0.0031–0.0064)	0.011(0.0050–0.018)	0.124(0.106–0.149)	0.165(0.132–0.258)	0.95(0.74–1.10)

TABLE 2—Continued

0.124	0.0064	0.063	0.197	0.275	1.26
0.109	0.0066	0.051	0.168	0.254	2.05
0.230	0.0041	0.008	0.150	0.269	1.08
0.159	0.0072	0.125	0.211	0.345	1.92
0.136	0.0097	0.109	0.246	0.353	3.13
0.233	0.0050	lost	0.299	1.536*	1.40
0.161(0.161–0.162)	0.0137(0.0118–0.0157)	0.207(0.181–0.232)	0.335(0.333–0.338)	0.454(0.424–0.485)	2.06(1.97–2.15)
0.140	0.0101	0.104	0.256	0.366	3.26
0.287(0.262–0.314)	0.0067(0.0054–0.0081)	0.011(0.0078–0.0164)	0.0263(0.242–0.276)	0.659(0.567–0.835)[3]	2.04(1.66–2.60)
0.164(0.158–0.171)	0.0074(0.0071–0.0077)	0.119(0.115–0.122)	0.276(0.258–0.293)	0.371(0.364–0.379)	2.79(2.65–2.94)
0.146	0.0110	0.144	0.276	0.393	3.53
0.276(0.235–0.297)	0.0074(0.0044–0.0108)	0.022(0.010–0.037)	0.286(0.238–0.371)	0.401(0.395–0.407)[4]	2.46(1.54–4.15)
0.173(0.165–0.181)	0.0142(0.0106–0.0178)	0.275(0.250–0.299)	0.380(0.369–0.391)	0.474(0.456–0.493)	4.19(3.57–4.81)
0.166	0.0139	0.160	0.349	0.492	4.47
0.277(0.277–0.278)[4]	0.0085(0.0076–0.0090)[3]	0.0258(0.021–0.039)	0.350(0.312–0.420)	0.559(0.447–0.705)	2.56(2.03–3.46)
0.184	0.0070	0.228	0.344	0.473	3.15
0.165	0.0137	0.192	0.344	0.485	4.41
0.125[5]	0.0075[5]	0.037(0.035–0.038)	0.366(0.340–0.393)	0.616(0.548–0.685)[5]	2.75(1.84–3.66)

TABLE 2—Continued

SPLEEN	STOMACH AND INTESTINES		SUPRARENALS	KIDNEYS
	Full	Empty		
grams	*grams*	*grams*	*grams*	*grams*
0.053(0.032–0.074)	1.123(1.015–1.284)	0.34(0.29–0.41)	0.0027(0.0022–0.0032)	0.125(0.111–0.138)
0.027		0.29	0.0041	0.143
0.026(0.020–0.032)	0.773(0.668–0.935)	0.43(0.35–0.53)	0.0038(0.0032–0.0045)	0.142(0.120–0.164)
0.048(0.042–0.059)	1.080(1.032–1.148)	0.38(0.32–0.46)	0.0027(0.0024–0.0030)	0.130(0.121–0.138)
0.029		0.31	0.0044	0.151
0.026(0.018–0.039)	0.875(0.597–1.163)	0.46(0.34–0.64)	0.0050(0.0041–0.0070)	0.167(0.136–0.223)
0.043(0.040–0.048)	1.114(0.837–1.463)	0.52(0.47–0.56)	0.0042(0.0032–0.0050)	0.153(0.127–0.168)
0.036		0.55	0.0052	0.179
0.057(0.034–0.108)	2.481(1.746–3.923)	0.83(0.70–1.05)	0.0074(0.0066–0.0092)	0.223(0.186–0.345)
0.041	1.033	0.59	0.0046	0.147
0.039		0.64	0.0055	0.188
0.053(0.045–0.067)	1.899(1.835–2.000)	0.88(0.76–1.07)	0.0070(0.0064–0.0074)	0.210(0.203–0.222)
0.054(0.044–0.073)	1.419(1.038–1.662)	0.51(0.41–0.58)	0.0041(0.0030–0.0048)	0.180(0.161–0.207)
0.042		0.78	0.0059	0.203
0.061(0.034–0.095)	2.092(1.748–2.504)	0.92(0.64–1.17)	0.0082(0.0070–0.0094)	0.235(0.205–0.266)
0.053(0.047–0.066)	1.437(1.038–1.814)	0.52(0.41–0.65)	0.0043(0.0030–0.0054)	0.179(0.168–0.190)
0.045		0.87	0.0062	0.211
0.071(0.029–0.129)	2.346(1.690–2.567)	1.12(0.89–1.74)	0.0097(0.0076–0.0128)[3]	0.261(0.241–0.328)

0.104	3.698	1.35	0.0086	0.342
0.082		2.07	0.0098	0.338
0.095	2.645	1.31	0.0161	0.374
0.201	6.708	2.20	0.0161	0.540
0.127		3.27	0.0140	0.483
0.101	4.148	1.38	0.0251	0.616
0.137(0.123–0.152)	4.863(4.004–5.722)	2.29(1.87–2.72)	0.0156(0.0134–0.0177)	0.610(0.582–0.638)
0.133		3.41	0.0137	0.502
0.212(0.136–0.379)	4.668(3.819–5.587)	2.06(1.89–2.41)	0.0204(0.0185–0.0248)	0.494(0.424–0.553)
0.343(0.307–0.379)	8.030(7.647–8.413)	3.30(2.82–3.79)	0.0143(0.0130–0.0156)	0.531(0.520–0.542)
0.146		3.71	0.0159	0.542
0.238(0.117–0.525)	5.729(3.990–8.496)	2.01(1.89–2.25)	0.0238(0.0172–0.0394)	0.583(0.416–0.920)
0.337(0.188–0.486)	8.599(7.756–9.442)	4.04(3.94–4.13)	0.0172(0.0156–0.0188)	0.804(0.761–0.846)
0.193		4.70	0.0175	0.687
0.191(0.177–0.221)	10.427(5.700–20.820)	2.66(2.20–3.09)	0.0154(0.0144–0.0160)	0.676(0.646–0.733)
0.295	8.107	2.95	0.0182	0.783
0.190		4.63	0.0201	0.677
0.211(0.193–0.229)	17.209(11.438–22.980)	2.82(2.55–3.08)	0.0205(0.0175–0.0236)	0.659(0.659–0.660)

TABLE 2—Continued

TESTES	EPIDIDYMI	OVARIES	HYPOPHYSIS	PINEAL BODY
grams	*grams*	*grams*	*grams*	*grams*
0.016(0.014–0.018)	0.0078(0.0068–0.0095)		0.0012(0.0011–0.0014)	0.00084(0.0007–0.0010)
0.041			0.009	
0.046(0.039–0.054)	0.015(0.012–0.020)		0.0016(0.0014–0.0019)	0.00079(0.0006–0.0009)
		0.0018(0.0011–0.0026)	0.0012(0.0011–0.0012)	0.00077(0.0007–0.0008)
		0.0031	0.0009	
		0.0033(0.0019–0.0047)	0.0016(0.0010–0.0020)	0.00076(0.0006–0.0010)
0.042(0.033–0.051)	0.015(0.013–0.016)		0.0016(0.0014–0.0018)	0.00087(0.0008–0.0010)
0.054			0.0011	
0.068(0.037–0.088)	0.017(0.0100–0.0254)		0.0017(0.0015–0.0019)	0.00073(0.0006–0.0008)
		0.0034	0.0016	0.0008
		0.0039	0.0011	
		0.0065(0.0056–0.0078)	0.0018(0.0017–0.0019)	0.0007(0.0006–0.0008)
0.039(0.033–0.044)	0.015(0.010–0.019)		0.0017(0.0014–0.0018)	0.00079(0.0006–0.0009)
0.060			0.0012	
0.059(0.045–0.070)	0.014(0.012–0.015)		0.0021(0.0019–0.0022)	0.00082(0.0006–0.0010)
		0.0028(0.0019–0.0040)	0.0016(0.0013–0.0020)	0.00069(0.0006–0.0008)
		0.0043	0.0012	
		0.0043(0.0022–0.0062)	0.0020(0.0017–0.0024)	0.00084(0.0006–0.0010)
0.139	0.026		0.0022	0.0012
0.130			0.0018	
0.118	0.023		0.0020	0.0012

		0.0108	0.0030	0.0012
		0.0080	0.0025	
		0.0203	0.0030	0.0010
0.394(0.378–0.411)	0.059(0.055–0.064)		0.0033(0.0031–0.0034)	0.0012(0.0011–0.0013)
0.321			0.0026	
0.228(0.119–0.297)	0.040(0.021–0.053)		0.0028(0.0026–0.0030)	0.0012(0.0011–0.0013)
		0.0090(0.0080–0.0100)	0.0031(0.0030–0.0031)	0.0013(0.0013–0.0013)
		0.0085	0.0028	
		0.0105(0.0085–0.0128)	0.0034(0.0029–0.0041)	0.0011(0.0010–0.0012)
1.046(0.952–1.141)	0.108(0.095–0.121)		0.0042(0.0031–0.0052)	0.0011(0.0010–0.0012)
0.704			0.0034	
1.053(0.934–1.099)	0.315(0.257–0.428)		0.0033(0.0028–0.0039)	0.00075(0.0007–0.0008)
		0.0114	0.0036	0.0008
		0.0095	0.0038	
		0.0091(0.0072–0.0110)	0.0040(0.0034–0.0045)	0.0008(0.0007–0.0009)

[1] Average of 5 individuals.

[2] Average of 6 individuals.

[3] Average of 3 individuals.

[4] Average of 2 individuals.

[5] Average of 1 individual.

* Pathological.

RATIO OF TAIL LENGTH TO BODY LENGTH

Immediately after death each rat was laid on its back and gently extended. The distance from the tip of the nose to the anus, and from the anus to the tip of the tail was carefully measured. From the measurements thus obtained individual ratios between tail length and body length were computed. The average of these tail ratios expressed as percentage of the nose-anus length is given in table 1 for each group of rats.

The data in table 1 show that with advancing age, the tail ratio increases in both the control and test rats. However, as compared with that in younger controls of corresponding body weight, the tail ratio, without exception, is much higher in the test rats. At ten weeks of age the ratio for the underfed individuals averages approximately 0.78 (73.9 per cent in the males and 82.0 per cent in the females), as compared with an average of 0.55 (53.7 per cent in the males and 56.6 per cent in the females) for the younger controls of corresponding weight. The differences at other ages, though somewhat less, are also very pronounced. It is therefore evident that in the underfed rats, the tail continues to grow in length more rapidly than the body. Thus the tail ratio in the underfed rats at ten weeks (0.78) approaches, but does not quite reach, the normal ratio of 0.88 (Jackson '15 b) for (larger) rats of corresponding age.

In the rats underfed for very long periods, however, the data (table 1) indicate an elongation of the tail (ratio 0.89–0.93) even beyond the normal ratio for corresponding age. A similar condition was observed by Jackson ('15 b) in two cases.

It may further be noted in table 1 that in the controls (except at one week of age), and also in the test rats, the tail in most instances averages relatively longer in the female than in the male. The longer tail in the female, as well as the marked elongation of the tail in young rats held at maintenance, is in agreement with the observations by Jackson ('15 b).

Hatai ('08) claimed that feeding rats upon an unsuitable diet of starch mixtures produced short-tailed individuals. Hatai ('15), however, found the tail length in five stunted rats fed upon a lipoid-free ration to exceed slightly the length given by the Wistar reference tables for normal rats of corresponding body length. Also in a later personal communication to Prof. C. M. Jackson he stated that in rats subjected to chronic inanition the tail becomes relatively long.

Harms ('09) observed that while starvation produced a marked decrease in the body length of Triton, the tail length remained practically unchanged. Morgulis ('11) however found the converse to be true in Diemyctylus.

Jackson ('15 a) found that acute and chronic inanition in adult albino rats also tends to produce relatively long-tailed individuals, due probably to a shrinkage in the trunk length.

HEAD

In the test rats underfed from birth to three and ten weeks of age, the absolute weight of the head (table 1) is slightly higher than in the controls of corresponding body weight. In the test rats at three weeks of age there is an apparent increase from an average of 2.42 grams for the younger controls (sexes combined) to 2.83 grams, an increase of approximately 16 per cent (uncorrected for slight difference in body weight). The increase in head weight at this age is due in large part to the increase in the weight of the brain. At ten weeks of age, the increase of the head weight in the test rats (about 4 per cent) is very slight. At six weeks of age, the weight of the head for the underfed individuals (3.27 grams) is slightly lower than the average for the younger controls (3.40 grams). In the rats underfed for very long periods the weight of the head exceeds that in the controls 16 to 26 per cent.

In general, therefore, the data indicate that in very young rats underfed for considerable periods, the weight of the head slightly exceeds that of the younger controls of corresponding body weight, especially after very long periods of underfeeding.

Jackson ('15 b) similarly found in the majority of instances the head apparently to increase slightly in weight in young rats kept at maintenance for various periods. In adult rats subjected to both acute and chronic inanition Jackson ('15 a) found the head to become relatively large, due to the very sight loss of the head in weight, as compared with the loss in weight of the body as a whole.

EXTREMITIES AND TRUNK

According to Jackson and Lowrey ('12) the upper extremities (fore limbs) at one week of age normally form an average of 8.92 per cent of the body, the average net body weight being 11.6 grams. In my controls of the same age, but somewhat lighter in weight (9.8 grams net), the average relative weight of the upper extremities (6.9 per cent) is lower. This difference may be due in part to variation in the technique used in removal of the extremities, though in both cases they were divided at the shoulder joint.

As compared with my controls the weight of the upper extremities (table 1) is heavier in the test rats underfed from birth to three weeks of age. The apparent growth from 0.678 to 0.800 gram (sexes combined) represents an increase of about 18 per cent. In the case of the rats underfed longer periods the weights of upper extremities are variable, but in the majority of instances they are lighter in the test animals than in the controls. On the whole, the differences are not very striking.

In the case of the lower extremities (table 1), the average relative weight of the hind limbs (9.53 per cent) for my controls at one week, is slightly lower than the relative weight (11.97 per cent) obtained by Jackson and Lowrey ('12) for normal rats of the same age.

As compared with my controls, the data in general indicate no distinct change in the weight of the hind limbs in the test rats. In the case of the four males underfed until 291 days of age there is an apparent increase from 11.58 grams in the controls to 14.20 grams in the test rats. The lower extremities are also much heavier in the females underfed to 314 days of age as compared with the younger control of corresponding body weight. At all other ages, however, the differences are variable and much less marked.

The weight of the trunk (table 1) is also variable, but in the majority of cases averages slightly lower in my test rats than in the controls. The differences, however, are small, and therefore, as in the case of the lower extremities, are of questionable significance.

On the whole, therefore, the data indicate that the weights of the extremities are practically normal in the test rats as compared with the younger controls of corresponding weight. The slight increase in the head, especially in the older groups, is apparently compensated for in most cases by a corresponding decrease in the weight of the trunk. Considerable allowance should be made, however, for experimental error and normal variability.

Jackson ('15 b) in somewhat similar experiments noted no distinct change in the body proportions. There was, however, an apparent slight increase in the head, counterbalanced by a corresponding decrease in the trunk and extremities. During inanition in adult rats, apparently both the head and the extremities increase in relative weight, whereas the trunk decreases (Jackson '15 a).

INTEGUMENT

In my young test rats underfed from birth to six and ten weeks of age, the weight of the integument (table 1) is considerably lower than in the controls. At 10 weeks of age the average decrease from 3.9 to 2.0 grams amounts to a loss of about 48 per cent. Jackson ('15 b) likewise observed that the integument suffers a considerable loss in weight in young rats held at maintenance for various periods. For my test rats underfed from birth to three weeks, however, the relative weight of the integument averages 21.13 per cent of the body weight, as compared with 21.05 per cent for the younger controls of corresponding body weight. Thus it is evident that in rats underfed from birth to three weeks there is practically no change in the weight of the integument, which is in marked contrast to the loss suffered in the test rats fasting for longer periods. The greater severity of the longer fasts as compared with the shorter period may account in part for the differences observed. This may be due to a stronger growth tendency in the integument during the early maintenance period, or to a stronger tendency to accumulation of fat in the normal integument in the later periods.

In the test rats underfed for very long periods and weighing between 30 and 50 grams, the weight of the integument averages still somewhat lower than in the controls of corresponding weight, although the difference is less marked than in the ten weeks' group. The weight of the integument in the test rats weighing approximately 70 grams at the end of long periods of underfeeding, however, averages considerably higher than in the corresponding controls.

Aron ('11) found the skin in dogs held at constant weight to show a slightly higher percentage of the body weight than in normal control dogs. In these experiments, however, as pointed out by Jackson ('15 b), if comparison had been made with the controls at the beginning of the experiment instead of at the end, the opposite conclusion might have been reached. During acute and chronic inanition in adult rats, Jackson ('15 a) found the integument to lose weight in nearly the same proportion as the whole body.

Although the integument as a whole in most cases suffers a marked loss in weight, it was nevertheless observed that the external ear (fig. 1) in the young stunted rats, steadily continues to increase in size, and also changes its form so as to approach the normal adult appearance. The continued growth of the external ear is probably associated with the growth tendency of its skeletal (cartilaginous) portion.

SKELETON

The ligamentous, moist cartilaginous and dry cartilaginous skeletons were prepared as described by Jackson ('15 b). The moist skeleton was dried to constant weight in an oven at 85°C.

It is evident from the data in table 1 that the weight of the ligamentous skeleton is constantly higher in the test rats than in the controls of corresponding weight. In the test rats at ten weeks of age there is an apparent increase in the weight of the ligamentous skeleton from an average of 2.357 grams (sexes combined) for the younger controls to 2.915 grams for the underfed individuals. The increase amounts to nearly 24 per cent (uncorrected for slight difference in body weight).

Similarly, the data for the moist cartilaginous skeleton (table 1) show a marked increase in the underfed rats. For the test animals ten weeks of age the average absolute weight of the moist cartilaginous skeleton (2.470 grams) exceeds that for the younger controls (1.597 grams) by 0.873 gram, an excess of about 54 per cent. At other ages the differences are also very striking.

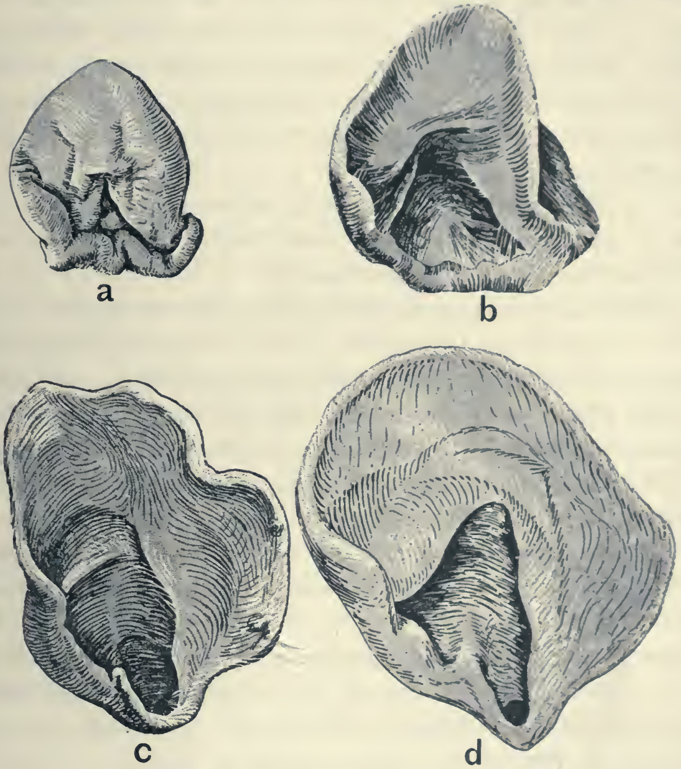


Fig. 1 a. External ear of a normal albino rat two weeks of age weighing 15.0 grams. × 4. b. External ear of an albino rat underfed from birth to three weeks of age. Weight of rat was 11.5 grams. × 4. c. External ear of an albino rat underfed from birth to ten weeks of age. Weight of rat was 15.4 grams. × 4. d. External ear of a normal albino rat ten weeks of age weighing 89.0 grams. × 4.

The weight of the ligaments and periosteum (obtained by subtracting the weight of the moist cartilaginous from the weight of the ligamentous skeleton) apparently has decreased considerably in the test rats at three, six and ten weeks of age. For the last group there is an apparent loss in weight from an average of 5.12 per cent of the body weight in the controls, to an average of 3.04 per cent in the test rats. The data for the ligaments and periosteum at the end of the very long fasting periods are variable, but in the majority of instances there is an indication of an increase in the weight of this portion of the skeleton.

Jackson ('15 b) in similar experiments starting at three weeks of age, found the ligamentous skeleton to manifest a marked growth tendency during maintenance.

The alkaline solution in which the skeletons are boiled probably acts more severely on the tender skeletons of my young controls than on the tougher and more calcified skeletons of the older test rats, thus giving an abnormally high apparent weight for the ligamentous portion of the skeleton in the controls. This would no doubt tend to mask any actual increase in the ligaments and periosteum in the rats underfed for the shorter periods. This may account for the difference between my results in earlier periods of fasting and those obtained by Jackson for the ligamentous structures.

In the case of the dried cartilaginous skeleton the data (table 1) indicate a greater percentage of dry substance in the skeletons of the test rats than in the controls. At ten weeks of age the percentage of dry substance amounts to 23.4 per cent of the moist cartilaginous skeleton in the controls and 30.3 per cent in the test rats. Also at other periods (both older and younger) the percentage of dry substance in the skeletons of the test rats exceeds that for the controls.

It is evident, therefore, that during the underfeeding the skeleton had gained in solids and lost in water content. Thus the continued growth of the skeleton had proceeded along the lines of normal development, for Lowrey ('13) finds the dry substance of the normal ligamentous skeleton to increase steadily from an average of 33.3 per cent at twenty days of age to 52.6 per cent at one year.

Further evidence of growth continuing according to the normal process is indicated by the appearance of the third molar in the mandible and maxilla of the test rats underfed from birth to ten weeks of age. This tooth is not visible in the jaws of the younger controls with the same body weight.

The various individual bones of the skeleton in the underfed young rats are not only larger but also more advanced in their stage of development. The epiphyses at the ends of the humerus, especially at the distal end, are well fused with the shaft, much more so than in the case of the younger controls. Also the epiphyses at the ends of the femur and tibia are more completely developed in the test animals at ten weeks of age than in the controls, and in the former only has the tibia fused with the distal end of the fibula.

In general, therefore, the results confirm the observations of Jackson ('15 b) that during maintenance of body weight in young animals, not only skeletal increase in mass occurs, but also skeletal growth and differentiation of apparently normal character, though somewhat retarded in rate.

Waters ('08), who found that calves continue to increase in height and width of hip for a considerable time when held at maintenance, was probably the first to observe the fact that the skeleton continues to grow in young animals even when the body weight is held constant.

Aron ('11) noted an increase in the length and height of young dogs held at constant weight for considerable periods. He also found an increase in nine of the individual bones, but made no observations upon the entire skeleton. The first complete and systematic study of the growth of the skeleton in young animals held at maintenance was by Jackson ('15 b).

Birk ('11), Aron ('14), Hess ('16) and others show that a strong growth tendency of the skeleton is apparently manifested also in children during malnutrition.

During acute and chronic inanition in adult rats the skeleton nearly maintains its original weight (Jackson '15 a).

MUSCULATURE

The absolute weight of the musculature (table 1) averages constantly higher (except in the four test males at 412 days, and in one female at 316 days) in the underfed rats than in the controls of corresponding body weight. The apparent increase in the individuals underfed from birth to ten weeks from 3.85 grams to 4.24 grams (sexes combined), however, represents an increase of only 10 per cent. The greatest excess is in the four test males at 291 days, in which the weight of the musculature exceeds that of the (younger) controls by about 20 per cent. At other ages, however, the differences are not very striking. In the exceptional cases mentioned above the average weight of musculature for the test rats is slightly below that for the (younger) controls of the same body weight.

In general, therefore, it appears that the musculature in the test rats differs only slightly from that of the controls. The averages, however, indicate a slight increase in weight in the majority of cases. On account of the difficulty in removing the musculature completely in a uniform manner, the exceptions may be due to experimental error, rather than to variability in the musculature.

Jackson ('15 b) likewise observed but slight change in the weight of the musculature in young rats kept at constant body weight, although in the majority of cases there was a slight tendency to increase. Since the tendency is even greater and more constant in my series, it may be concluded with a fair degree of certainty that in young rats of various ages held at maintenance for various periods the musculature shows a slight but definite tendency to increase in weight in the great majority of cases.

During both acute and chronic inanition in adult rats, Jackson ('15 a) found the musculature to lose relatively in nearly the same proportion as the whole body, the loss being somewhat greater in chronic than in acute inanition.

VISCERA

The weight of the visceral group as a whole (table 1), including the abdominal and thoracic viscera, brain, spinal cord and eyeballs, in the rats underfed from birth to three, six and ten weeks of age, average constantly higher than in the corresponding controls. The increase in absolute weight amounts to approximately 28, 29 and 38 per cent in the test rats at three, six and ten weeks respectively. In the rats underfed from 21 to 412 days of age the increase is less marked, while in the test rats weighing 75 grams at 291 days there is an apparent decrease in the weight of the visceral group from 13.68 to 11.19 grams, a loss amounting to approximately 19 per cent.

From the foregoing, it is evident that in rats underfed from birth to three, six and ten weeks of age, there is a distinct increase in the weight of the visceral group. In the older test animals the changes are less marked, in some instances actually showing a considerable loss in weight. My data, however, indicate an increasing tendency to loss of weight in the visceral group in prolonged retardation of body growth, although the individual viscera differ in this respect, as will appear later.

Jackson ('15 b) likewise found the visceral group to show a distinct increase in weight in rats kept at maintenance from three to six, eight and ten weeks of age, but found no essential change in the later periods. According to Jackson ('15 a) the visceral group undergoes little change in relative weight in adult rats during acute and chronic inanition.

'REMAINDER'

The weight of the 'remainder' (table 1), which includes some small unweighed organs, fat, and body-fluids, was obtained by deducting the weight of the integument, skeleton, musculature and viscera from the net body weight. The intestinal contents are therefore not included.

In the rats underfed from birth to three weeks of age the 'remainder' has apparently suffered an average decrease from 1.843 to 1.092 grams or a loss of approximately 40 per cent, which was compensated for by an increase in the skeleton and musculature. The marked decrease of the 'remainder' in these very young rats may possibly be due to a decrease in body fluids, the percentage of which is very high in young rats (Jackson and Lowrey '12), (Lowrey '13).

In the later periods the data show considerable variability in the 'remainder,' but in most instances the weight of the 'remainder' is higher in the later periods.

Jackson ('15 b) found considerable variability in the 'remainder' of young rats held at maintenance beginning at three weeks or later, but considered it doubtful whether there is any definite change in the test rats, as compared with the normal. In adult rats subjected to acute or chronic inanition, however, Jackson ('15 a) found a definite decrease in the weight of the 'remainder,' probably due to loss of fat.

BRAIN

The weight of the brain (table 2) in the test rats underfed from birth to three weeks of age greatly exceeds that in either the corresponding controls or the Wistar norm for rats of the same body length as the controls. There is an increase from an average (sexes combined) of 0.616 gram for my controls to 0.985 gram in the test rats. This is an absolute increase of 0.369 gram, or a relative increase of about 60 per cent (uncorrected for slight differences in body weight). The average relative weight (9.80 per cent) of the brain in this group of test rats greatly exceeds the normal maximum of about 6.7 per cent of the body weight observed by Jackson ('13) for rats weighing approximately 15 grams.

In the rats underfed for longer periods the weight of the brain is more nearly equal in test rats and controls. As shown in table 2, however, even in these groups the test rats (excepting the females at 42 days) slightly exceed the controls in absolute brain weight. The maximum apparent increase amounts to 13.3 per cent in the female at 316 days, and the average increase is about 5.8 per cent. This is subject to a small correction on account of slight differences in body weight. It may also be noted that the brain in my controls is usually heavier than the Wistar norm for rats of corresponding body length.

From the foregoing it is evident that in rats underfed from birth to three weeks of age there is a marked increase in the weight of the brain, whereas during the longer fasting periods (both in those beginning at birth and those beginning at three weeks) the increase is very slight. Bechterew ('95) found acute inanition in puppies and kittens to produce an apparent loss of weight in all of the organs, including to a slight extent the central nervous system. The greatest loss suffered by the central nervous system was apparently in the cerebral hemispheres, whereas the smallest loss was in the spinal cord. Microscopic examination revealed evidence of delayed medullation in fiber tracts not fully developed when the fasting started.

Hatai ('04) noted an apparent absolute decrease of 5 per cent in the weight of the brain in young rats suffering a loss of approximately 30 per cent in body weight due to an unfavorable diet of starch and beef-fat.

Later, Hatai ('08) found the brain weight in rats stunted by feeding upon an unfavorable diet, to be practically identical with that for normal younger rats of the same body weight.

Donaldson ('11) found the weight of the brain in a large series of rats held nearly at maintenance from 30 to 51 days of age to average 7.7 per cent less than that for the full-fed controls at the same age. However, he points out that if comparison be made with the calculated initial brain weight, an increase of 3.6 per cent is apparent in the underfed rats. This in general agrees with the results of the present investigation.

Jackson ('15 b) observed practically no change in the weight of the brain in young rats held at maintenance for various periods starting at three weeks of age. My experiments started on much younger rats, and during a period when the brain normally shows a marked growth capacity. This probably accounts for the remarkable increase in brain weight observed for my youngest group. At later periods the brain loses its earlier intensity of growth, which is more nearly equalled by the remainder of the body. During acute and chronic inanition in adult rats the brain loses little if any in absolute weight (Jackson '15 a).

SPINAL CORD

The weight of the spinal cord (table 2) like the brain in my controls averages slightly higher than the Wistar norm for rats of corresponding length, the only exceptions being the two control males and one control female weighing about 70 grams net. The differences are less marked when the body weight is used as a basis for comparison. As compared either with my controls or with the Wistar tables, the spinal cord is constantly heavier in the test rats. In the test rats at three and ten weeks of age, the average increase (sexes combined) amounts to approximately 70 per cent, and at six weeks to about 38 per cent. In the other groups of test rats underfed for long periods, the increase while still considerable, is somewhat less marked, varying from 29 to 56 per cent in the various groups (table 2).

Donaldson ('11) also found an increase in the weight of the spinal cord in rats held at 34 grams body weight from 30 to 51 days of age.

Jackson ('15 b) found that during maintenance there was a well-marked increase in the weight of the spinal cord, amounting to 36 per cent in those at maintenance from three to ten weeks of age.

It therefore appears that in young rats underfed at various ages the spinal cord shows a remarkable tendency to grow, this tendency being relatively strongest in those stunted from birth. This increase in weight is also maintained (to a lesser degree) in young rats underfed through long periods of time.

According to Jackson ('15 a) the spinal cord suffers practically no loss in absolute weight during acute and chronic inanition in adult rats.

EYEBALLS

The weight of the eyeballs in my controls (table 2) averages constantly higher than the Wistar norm for rats of corresponding length. The differences, however, are not striking and are probably due largely to differences in body weight (my rats averaging slightly heavier than the Wistar norm for corresponding length).

When the weight of the eyeballs for the controls is compared with the weight for the test rats, it is evident that a marked increase has occurred in the latter. At ten weeks of age the increase in weight from an average of 0.093 gram in the controls to 0.155 gram in the test rats, equals an increase of more than 66 per cent. At six and ten weeks of age the excess weight in favor of the test rats is less, being about 41 and 34 per cent respectively. The increase at later periods, in some instances is even more striking, amounting to approximately 73 per cent (sexes combined) in the rats weighing nearly 50 grams after very long periods of fasting.

My results, therefore, confirm Jackson's ('15 b) observation that the eyeballs show a marked growth capacity in young rats kept at maintenance for various periods. He suggests that the possibility of continued growth of the eyeballs is due largely to water absorption, the water content of the eyeballs being normally very high (85.6 per cent at 20 days according to Lowrey' 13).

The eyeballs suffer practically no loss in weight during acute and chronic inanition in adult rats according to Jackson ('15 a).

A few observations were made concerning the time at which the eyelids opened in my albino rats. The data (table 3) indicate that the eyelids opened in my controls at an average age of fifteen days, whereas in the test rats the opening is apparently delayed until about the seventeenth day. The body weight of the controls when the eyelids opened averaged 16.5 grams as compared with 9.9 grams for the test rats. Therefore, although somewhat retarded in time, the eyelids nevertheless opened at a lighter body weight in the underfed individuals than in the controls. However, in the case of one litter (St 63) it was noted that the eyelids opened in two test rats weighing an average of 12.2 grams even at a younger age (15 days) than in the control. The exact age at which the eyes opened in the controls in this instance was not recorded except that it occurred after the fifteenth day at a body weight of more than 15.5 grams.

Bechterew ('95) likewise found a delay in the opening of the eyelids in newborn dogs and cats subjected to acute inanition.

Several investigators have noted the age at which the eyes normally open in the albino rat. Jackson ('12) states that the eyes are opened at about sixteen or seventeen days of age. Donaldson ('15) ('The Rat,' p. 19) states that the eyes open from the fourteenth to the seventeenth days, most often on the fifteenth or sixteenth.

King ('16) states that usually the female rats in a litter open their eyes several hours sooner than do the males. The delay in the opening of the eyelids observed in my test rats apparently is not because the data, include a large majority of females, for in practically every instance (see exception noted) the eyelids opened two or three days later in the test females than in the female controls of the same litter.

TABLE 3

Age and gross body weight at which the eyelids opened in the control and tests rats

CONTROLS				TEST RATS		
Litter number	Number of individuals and sex	Age in days	Gross body weight	Number of individuals and sex	Age in days	Gross body weight
			grams			*grams*
St 56	1(F)	15	16.0	3(1 M, 2 F)	18	7.9
St 59	1(F)	15	11.3	8(1 M, 7 F)	18	8.6
St 63	1(F)	15+	15.5+	2(F)	15	12.2
St 68	1(M)	17	20.0	1(F)	18	14.0
St 92	2(1 M, 1 F)	15	20.0	2(1 M, 1 F)	16	11.0
St 95				1(M)	18	11.0
St 102	1(F)	14	15.0	6(F)	17	10.2
St 106	1(F)	14	15.0	2(1 M, 1 F)	19	10.5
St 114	2(1 M, 1 F)	15	15.8			
Average*.	10(3 M, 7 F)	15	16.5	25(5 M, 20 F)	17.5	9.9

* Control of Litter St 63 excluded from the averages, as the exact time at which the eyelids opened was not recorded.

THYROID GLAND

In the test rats fasting from birth to three, six and ten weeks of age the weight of the thyroid gland (table 2) is practically identical with that in the controls. However, in the rats underfed for very long periods, in which the experiments started at three weeks of age, the weight of the thyroid in most instances shows a marked decrease in the test rats when compared with either my controls or with the Wistar norm tables. For example, in the case of the eight test rats weighing about 50 grams after very long underfeeding the thyroid has apparently suffered a loss of approximately 50 per cent in the males, although there is apparently no change in the females.

These latter results therefore agree in general with those obtained by Jackson ('15 b) in rats held at maintenance from the age of three to six, eight and ten weeks. In my younger rats, however, subjected to inanition for various periods starting at birth, the weight of the thyroid apparently remains practically unchanged. The power of maintenance in the thyroid therefore appears stronger in the very young rats. Considerable allowance should be made for experimental error due to the difficulty in dissecting the thyroid gland in an accurate manner.

During acute inanition in adult rats, Jackson ('15 a) reports that the thyroid gland apparently loses little or no weight, while in chronic inanition with an average loss in body weight of 36 per cent, the thyroid gland loses only about 22 per cent in weight.

THYMUS

As is evident from table 2, the weight of the thymus is, without exception, much lighter in the test rats than in the controls. At three, six and ten weeks of age the loss in weight amounts to approximately 30, 60 and 80 per cent respectively. The decrease in weight of the thymus in the test rats, while very marked in all groups, is especially striking in the four test males underfed 412 days. From an average of 0.207 gram in the controls, the weight in the test rats has decreased to 0.011 gram, a loss of approximately 94 per cent. This decrease is not so remarkable, however, when the normal involution with age is taken into account. According to the Wistar tables, the normal weight of the thymus at 400 days is only 0.039 gram.

Thus it is evident, as was observed by Jackson ('15 b) that the thymus loses markedly in weight in young rats underfed for various periods. He found a loss of about 90 per cent for the thymus in rats held at maintenance from the age of three to ten weeks.

Jonson ('09) likewise found in young rabbits kept at constant body-weight for four weeks the weight of the thymus to be reduced to about one-thirtieth its initial value. The greatest loss is suffered by the cortex, which is reduced to one-twelfth of its initial weight during two weeks of maintenance. Judging from comparison with my own results, as well as with those of Jackson, the process of hunger involution of the thymus would appear to be much more rapid and complete in the rabbit than in the rat.

HEART

The weight of the heart (table 2) in the rats underfed from birth to three weeks of age differs but little from that in the controls (decrease of about 10 per cent in the males; slight increase in the females). At six and ten weeks of age, however, there is an evident increase in the heart weight. In those underfed from birth to six weeks, the average increase is about 13 per cent. For the groups underfed to ten weeks, the increase from an average of 0.092 gram in the controls to 0.117 gram in the test rats (sexes combined) represents an increase of about 27 per cent (subject to slight correction for differences in body weight). In the rats underfed for very long periods, the heart weight, while variable in most instances, is lower than in the controls. There is a decrease in the various groups, varying from about 8 to 29 per cent, excepting the four females at 392 days, which show an apparent increase of about 3 per cent (most of which may be accounted for as due to difference in body weight).

In general, therefore, the data indicate a slight increase in the weight of the heart in the test rats at six and ten weeks of age, while during the later periods the heart apparently lost weight in the majority of instances. However, on account of the normal very considerable variability of the heart weight, especially in young rats (Jackson '13), the apparent changes in the heart during underfeeding are probably somewhat doubtful.

In young rats kept at constant body weight for various periods, and also in adult rats subjected to acute and chronic inanition Jackson ('15 a, '15 b) found the percentage weight of the heart to remain practically unchanged. Apparently the effects were less marked than in my experiments, in which the underfeeding was begun earlier, or carried over longer periods.

LUNGS

As compared with the Wistar norm for rats of corresponding body length, the weight of the lungs (table 2) appears to be unusually high in my young controls. For the largest group (14 rats, 7 males and 7 females, 13 days old) weighing about 15 grams, the average absolute weight of the lungs (sexes combined) is 0.234 gram, as compared with the Wistar average of 0.166 gram for rats of corresponding body length.

Jackson ('13) found the normal weight of the lungs in the rat at one week to average 0.1938 gram, with which my average of 0.1995 gram for controls of the same age agrees closely. It appears, therefore, as pointed out by Jackson ('13), that weights for the lungs derived from Hatai's formula appear much lower than the actual observed values in very young rats.

As compared with my controls, the average weight of the lungs is low in the test rats at three, six and ten weeks of age, the decrease in each case amounting to a loss of approximately 26 per cent (subject to slight correction for difference in body weight).

In the longer experiments the lungs average heavier for the underfed rats than in the controls, with the exception of one female at 139 days. The presence of lung infection among the older underfed rats probably accounts in part at least for the increase in the weight of the lungs. The data for the test rats weighing about 70 grams after very long fasting also include the weights of the lungs for five rats which were asphyxiated. In these instances the weights would no doubt be high due to the greater amount of blood retained in the lungs. Therefore the effect of the very long fasting period upon the weight of the lungs is doubtful.

Jackson ('15 b) found that in young rats subjected to maintenance for various periods, the lungs show a slight decrease in weight during the earlier periods (decrease of 15 per cent) but not later. It is evident from my results that the loss in the lung weight in very young underfed rats is considerably greater.

During acute and chronic inanition in adult rats the lungs lose weight in about the same proportion as the whole body (Jackson '15 a).

LIVER

The weight of the liver (table 2) in my young control rats is very much lower than the Wistar norm for rats of corresponding body length. The differences are especially marked in the controls weighing approximately 13 and 15 grams. My average value of 0.37 gram at one week, however, corresponds rather closely with the weight of 0.3431 gram obtained by Jackson ('13) in rats of the same age and weight. Thus, as pointed out by Jackson, it seems that weights for the liver obtained by Hatai's formula, especially for young rats, are much too high, at least for rats of the Missouri and Minnesota colonies. The difference appears too great to be attributed to normal variability or to slight differences in diet. However, C. Watson ('10) finds a marked decrease in the relative size of the liver in captured wild rats fed upon bread and milk diet used during captivity; and Jackson ('13) has emphasized the marked and irregular variability found in the liver of the rat.

In the rats underfed from birth to three, six and ten weeks of age the weight of the liver is considerably higher than in the corresponding controls. The increase at three weeks averages about 17 per cent. The difference is especially marked, however, at six and ten weeks, amounting to an average increase of about 83 per cent in the former and 64 per cent in the latter (subject to correction for slight differences in body weight).

In the rats underfed for longer periods the liver is variable. At 412 days of age the average weight of the liver (2.04 grams) in four test males is practically identical with that in the two controls (2.06 grams). The liver weight of the controls appears to be abnormally low, however. In the remaining groups of test rats underfed for very long periods the weight of the liver is considerably lower than in the corresponding controls. At 291 days of age there is an apparent decrease in the weight of the liver from 4.19 grams to 2.56 grams, a loss of about 39 per cent (uncorrected for slight difference in body weight).

In general therefore the data indicate that in young rats underfed for various periods, the liver shows a marked growth tendency in the very young rats, while in the very long fasting experiments there is usually a marked decrease in the weight of the liver.

Jackson ('15 b) likewise found that in young rats (three weeks old or more) held at maintenance the liver increases slightly (about 10 per cent) in weight during the shorter fasting periods; but in experiments extending over longer periods there is a decided decrease in the liver weight.

During inanition in adult rats, the liver loses in weight relatively more than the whole body, and to a greater extent in acute than in chronic inanition (Jackson '15 a).

SPLEEN

From table 2 it is very evident that in my young controls weighing approximately 10, 13 and 15 grams, the weight of the spleen greatly exceeds the Wistar norm for the spleen in rats of corresponding body-length. This is especially true for the youngest group. However, my average weight for the spleen (0.051 g.) at one week agrees more nearly with Jackson's ('13) normal spleen weight (0.0425 g.) at the same age. It has been noted by Jackson ('13) that the weight of the spleen derived from Hatai's formula is in general somewhat too low, probably because Hatai (without apparent justification) excluded all 'enlarged' spleens from his data.

The weight of the spleen (table 2) at three weeks of age is considerably lower in my test rats than in the controls. In this group there has been an apparent decrease from 0.051 to 0.026 gram, a loss of about 49 per cent (uncorrected for slight difference in body weight).

In the rats underfed from birth for longer periods (up to six and ten weeks), however, the spleen shows a marked tendency to growth, the average apparent increase being about 27 per cent.

In rats underfed for longer periods, beginning at three weeks of age, on the contrary, there is an apparent decrease in the weight of the spleen, except in the four females at 412 days. On account of the extreme normal variability of the spleen, however, the apparent atrophy during the later fasting periods may be of doubtful significance. It is nevertheless in agreement with the doctrine that lymphoid tissues in general tend to atrophy during inanition (cf. Jolly et Levin, '11).

Jackson ('15 b) noted a reduction in the weight of the spleen in young rats during the earlier periods of underfeeding (beginning at three weeks), while at later periods there was no material change in weight. In adult rats during chronic inanition the average loss in weight is nearly proportional to that of the entire body while in acute inanition the loss appears very much greater (Jackson '15 a).

STOMACH AND INTESTINES

The average weight of the stomach and intestines (including contents) is 1.10 grams (table 2) for my controls at one week. This is somewhat higher than the normal weight 0.733 gram obtained by Jackson ('13) for a large series of the same age. The difference is not entirely due to a difference in the amount of the contents, for the weight of the empty alimentary canal (0.36 gram) likewise averages higher than Jackson's weight (0.296 gram).

The weight of the contents of the alimentary canal (obtained by deducting the weight of the empty from the full alimentary canal) is much lower at three weeks in the test rats than in the controls. At six and ten weeks of age, however, the weight of the contents is excessively heavy in the underfed rats. In the later periods of underfeeding the data are variable but show a tendency to decrease.

Jackson ('15 b) noted an increase in the contents of the alimentary canal during the early periods of underfeeding, while during the longer experiments the change (a tendency to decrease) was less marked. This is in agreement with my findings, excepting the youngest group.

As compared with my controls, the weight of the empty alimentary canal is higher in the test rats at three, six and ten weeks of age than in the controls. At ten weeks of age the weight of the empty digestive canal apparently has nearly doubled in the underfed animals.

At the later periods, however, the data indicate a reduction in weight of the empty alimentary canal, which is especially striking (34 per cent decrease) in the 4 males weighing about 75 grams at 291 days of age.

Jackson ('15 b) likewise found the empty alimentary canal to increase in weight during the shorter maintenance periods, after which there was a decline in weight.

During both acute and chronic inanition in adult rats there is a very marked decrease in the weight of the stomach and intestines, both with and without contents (Jackson '15 a.).

SUPRARENAL GLANDS

The average weight of the suprarenals (table 2, sexes combined) (0.0027 g.) for my controls at one week agrees fairly with the normal average (0.00226 g.) obtained by Jackson ('13), but is far below the weight given in the Wistar tables for rats of corresponding body length. Thus the weights for the suprarenals at one week derived from Hatai's formula seem much too high, at least for normal rats from the Missouri and Minnesota colonies. For the controls at 13 and 14 days of age (table 2) the difference is less marked.

As compared with my controls, the weight of the suprarenals (except in the four males at 291 days) is constantly higher in the test rats. The average increase in the weight of the suprarenals (sexes combined) in the test rats at three and six weeks of age amounts to more than 60 per cent (uncorrected for slight difference in body weight). At ten weeks of age the difference has reached a maximum average of approximately 114 per cent. At later periods (except in the four males at 291 days, in which instance there was a loss of about 11 per cent) the suprarenals in the test rats exceed those of the corresponding controls 12 to 87 per cent. It should be noted that the weight of the suprarenals is slightly higher in the test females than in the males of corresponding body weight at ten weeks of age and also at all subsequent periods, thus indicating the appearance of sexual differentiation during underfeeding.

Jackson ('15 b) also found the suprarenals to manifest a marked growth tendency in young rats held at maintenance. He noted a maximum increase of 39 per cent in the female underfed from three to ten weeks of age. Apparently the growth of the suprarenals was less marked than in my experiments, in which the underfeeding was begun earlier or carried over longer periods. As occurs normally, the suprarenals during underfeeding underwent sexual differentiation in weight, also confirming the results of Jackson. In adult rats, there is but little loss in the absolute weight of the suprarenal glands during inanition (Jackson '15 a).

KIDNEYS

Using my controls as a basis for comparison, there is apparently an increase in the weight of the kidneys (table 2) in the rats underfed from birth to three, six and ten weeks of age. The apparent average increase (uncorrected for difference in body weight) amounts to about 21 per cent at three weeks, 45 per cent at six weeks, and 38 per cent at ten weeks.

In the rats underfed for very long periods the weight of the kidney is variable, some showing an increase and others a decrease. Due to this inconstancy in results, the changes are of questionable significance.

In general therefore it appears that, as was observed by Jackson ('15 b) the kidneys tend to increase in weight during the earlier periods of the experiment, but show no definite change during later periods. The increase in my young rats is more striking than the increase observed by Jackson. This is probably due to the fact that my rats were placed upon the experiment at a younger age, when the intensity of growth in the kidney is greater.

In adult rats during acute and chronic inanition Jackson ('15a) found the kidneys to lose weight relatively slightly less than the entire body.

TESTES AND EPIDIDYMIDES

From the data in table 2 it can be seen that the Wistar data for the testes greatly exceed the corresponding weights for my control rats at 7 and 13 days of age. At one week the difference (0.016 to 0.041 gram) is especially striking. However the combined average weight of the testes and epididymides (0.0238 g.) for my controls at one week corresponds closely with Jackson's ('13) normal (0.0273 g.) at the same age. It is therefore apparent that the Wistar data are much too high for these young rats. For the older controls, my data correspond fairly well with the Wistar tables.

Using my controls for comparison the weight of the testes in the test rats is exceedingly high at three, six and ten weeks of age. At three weeks the weight of the testes in the test rats exceeds that for the younger controls of the same body weight approximately 188 per cent. At six and ten weeks of age the increase (uncorrected for differences in body weight) amounts to about 62 and 51 per cent respectively, having become progressively less.

In the rats weighing 75 grams at 291 days the weight of the testes is practically identical with that for the controls. At 139 and 412 days of age, however, the testes have evidently suffered a marked loss in weight. The decrease (0.394 gram to 0.228 gram) in the test males of the latter group amounts to a loss of about 42 per cent.

From the foregoing it is evident that in young rats the testes increase considerably during the shorter periods of underfeeding. With very prolonged stunting, however, the testes may show practically no change, or a loss which varies up to a considerable amount. Jackson ('15 a) noted an increase of 34 per cent in the weight of the testes in young rats held at constant body weight from three to ten weeks of age. My results are much more striking, however, which is probably due to the stronger growth tendency of the testes in my very young rats which were underfed from birth. Considerable caution must be exercised in drawing conclusions, however, on account of physiological variability in the weight of the testes.

The weight of epididymides (table 2) in my test rats of three and six weeks of age is higher than for the corresponding controls. For the youngest group there is an apparent increase from a normal weight of 0.0078 gram to 0.0154 gram an increase of over 95 per cent (uncorrected for slight difference in body weight). At six weeks of age the excess weight for the epididymides in the test rats is only 13 per cent.

In the rats underfed to ten weeks of age and also in those at 412 days, there is an apparent loss in the weight of the epididymi. The loss at ten weeks amounts to approximately 6 per cent, and at 412 days to about 32 per cent. However, in the test rats weighing 67.2 grams net at 291 days there is an apparent increase in the weight of the epididymi from 0.108 to 0.315 gram. In all cases the percentage losses above estimated are subject to correction for slight difference in body weight.

In general, therefore, the data indicate that in the youngest rats there is a marked increase in the weight of the epididymides, but that later there is a loss of weight in most cases.

Jackson ('15 b) found an apparent slight loss in the epididymides in rats held at constant body weight from three to six and ten weeks of age. In adult rats during inanition Jackson ('15 a) found that the testes and epididymides apparently lose weight in about the same proportion as the entire body.

OVARIES

The weight of the ovaries (table 2) in my controls at seven days of age (0.0018 g.) is very much lower than the Wistar norm (0.0033 g.) for corresponding body length. The difference amounts to more than 80 per cent. Jackson ('13) found the weight of the ovary to average 0.00121 g. at one week of age, which is even lower than my data. It therefore appears fairly certain that the weight of the ovary calculated from Hatai's formula is too high for this early period. At later ages my data do not differ so greatly from the Wistar tables.

In my rats underfed from birth to three, six and ten weeks of age, there is apparently a considerable increase in the weight of the ovary. At ten weeks the increase from an average of 0.0028 gram in the controls to 0.0043 gram in the test rats represents a gain of approximately 54 per cent (uncorrected for body weight). At three and six weeks of age the differences are greater, amounting to 83 and 91 per cent respectively.

The data for the ovaries in the females subjected to very long periods of fasting are variable, but in most instances show a tendency to increase. Conclusions, however, are hazardous on account of the small number of observations and also on account of the extremely great cyclic changes in the weight of the ovary due to ovulation. Also the normal growth curve of the ovary is very complicated, as shown by Hatai ('13).

Jackson ('15 b) observed in the ovaries a decrease of about 27 per cent in weight in young rats kept at constant body weight from three to ten weeks of age. His data, however, indicate a slight increase in the youngest group underfed from three to six weeks. My results would indicate that at earlier periods the growth impulse of the ovary in underfed rats is still stronger, resulting in a marked increase in its weight.

HYPOPHYSIS

The weight of the hypophysis (table 2) appears higher in my controls (except for the one female weighing about 70 grams) than the Wistar norm for rats of corresponding body length. The high weight for the gland in my controls can be attributed partly to differences in body weight, my rats being slightly heavier than the norm for corresponding body length.

As compared with my controls, the weight of the hypophysis at three, six and ten weeks of age is higher in the test rats than in the controls. At three and ten weeks of age the increase (sexes combined) for the test rats amounts to approximately 33 and 24 per cent respectively (uncorrected for slight difference in body weight). At six weeks of age the increase is less, amounting to about 8 per cent. There is apparently no sexual difference in the increase at these ages.

In the test rats underfed for very long periods, however, the hypophysis is variable. In the test males at 412 days there is an apparent average decrease in absolute weight from 0.0033 gram to 0.0028 gram, a loss of over 15 per cent. The decrease in the males at 291 days of age amounts to approximately 21 per cent. On the other hand, in the females fasting for long periods there is no such decrease. In fact for the one female at 316 days the weight of the hypophysis shows no change, whereas at 392 and 314 days there is an apparent average increase of approximately 10 and 11 per cent respectively (subject to slight correction for difference in body weight). It should be noted that the weight of the hypophysis is slightly higher in the very old test females than in the males, suggesting that sexual differentiation in weight has occurred.

In general, therefore, it appears that the hypophysis increases considerably in weight in underfed rats during the earlier periods. In the very long experiments however the sexes appear to react differently. The weight of the hypophysis in the males apparently suffers a loss during very prolonged fasting, but in the females it shows a slight increase in weight. Jackson ('15 b) similarly noted a distinct tendency to an increase with the appearance of sexual differentiation in the weight of the hypophysis in rats held at constant weight from three to ten weeks of age. During inanition in adult rats the relative weight of the hypophysis is practically unchanged (Jackson '15 a).

Quite recently Jackson ('17) has studied the volume-changes in the lobes of the hypophysis in rats underfed for various periods. In young rats kept at constant body weight the pars anterior is somewhat reduced in relative size, the intermedia and nervosa becoming correspondingly larger.

PINEAL BODY

The weight of the pineal body (table 2) is practically normal in each group of test rats when compared with the corresponding controls. The slight differences which exist (especially in some of the older groups) are of doubtful significance, especially when the exceedingly small size of the gland is taken into consideration.

Considerable allowance must be made for experimental error, and for variability (the extent of which is unknown).

The data for the control rats indicate no sexual difference in the weight of the gland at the ages observed. Hoskins ('16) likewise found no significant difference according to sex.

DISCUSSION

In general, the results of the present investigation concerning the effects of underfeeding upon very young rats and upon slightly older rats underfed for very long periods agree fairly well with those obtained by Jackson ('15 b) in rats held at maintenance for various periods beginning at three weeks of age. There are, however, certain differences found in my rats in which the underfeeding began shortly after birth, or was prolonged for very considerable periods. These differences are probably due chiefly to the varying tendencies to growth and maintenance among the various organs at different periods.

With reference to their growth tendency in young rats held at constant body weight, the organs are divided by Jackson ('15 b) into three classes: (1) those having a growth tendency so strong that they continue to increase, even when the body weight is held constant; (2) those which approximately hold their weight constant (variation within 10 per cent) under these conditions; and (3) those which are unable to maintain themselves and lose in weight.

The distribution of the organs according to this scheme for my rats underfed from birth to ten weeks, also for those weighing about 50 grams after very long periods of fasting, together with Jackson's ('15 b) results for rats underfed from three to ten weeks are shown in table 4.

On comparing my results with Jackson's it appears that in very young rats underfed from birth to ten weeks of age a larger number of organs show a tendency to increase than in rats underfed from three to ten weeks. On the other hand, very long fasting apparently adds to the number of organs which fall into the group showing approximate maintenance or a tendency toward loss in weight.

TABLE 4

Comparison, of growth tendency; (1)in young rats underfed from birth to ten weeks; (2) in young rats held at maintenance from three to ten weeks; and (3) in rats weighing approximately 50 grams after very long fasting

	MARKED TENDENCY TO GROWTH DURING UNDERFEEDING	PER CENT OF CHANGE	WEIGHT NEARLY CONSTANT DURING UNDERFEEDING	PER CENT OF CHANGE	MARKED LOSS IN WEIGHT DURING UNDERFEEDING	PER CENT OF CHANGE
Rats underfed from birth to ten weeks of age (Stewart)	Suprarenals	+114	Musculature	+10	Thymus	−80
	Alimentary canal	+100	Brain	+ 8	Integument	−48
	Spinal cord	+70	Thyroid	+ 4	Lungs	−26
	Eyeballs	+66	Epididymi	− 6		
	Liver	+64				
	Ovaries	+54				
	Testes	+51				
	Kidneys	+38				
	Heart	+27				
	Spleen	+24				
	Hypophysis	+24				
	Skeleton	+24				
Rats held at constant body weight from three to ten weeks of age (Jackson)	Eyeballs	+50	Liver	+10.3	Thymus	−90
	Spinal cord	+36	Kidneys	+ 4.1	Spleen	−42
	Testes	+34	Musculature	+ 3.0	Integument	−36
	Skeleton	+28	Brain	− 0.5	Ovaries	−27
	Alimentary canal	+28	Heart	− 0.6	Thyroid	−24
					Lungs	−15
	Suprarenals M	+12				
	F	+39				
	Hypophysis M	+18				
	F	+19				
Rats weighing about 50 grams after very long periods of fasting (twenty-one to about four hundred days)(Stewart)	Eyeballs	+73	Lungs	+28(?)	Thymus	−90
	Spinal cord	+40	Hypophysis, F	+10	Testes	−42
	Suprarenals M	+31	Brain	+ 4	Epididymi	−32
	F	+66	Musculature	+ 4	Alimentary canal	−27
	Skeleton	+32	Spleen	− 5		
	Ovaries	+17	Kidneys	− 6	Thyroid	−36
			Liver	− 7	Hypophysis M	−15
			Heart	−10	Integument	−11

Thus the liver, ovaries, kidneys, heart and spleen show a marked tendency to increase in weight in the rats underfed from birth to ten weeks, but not in those underfed from three to ten weeks. On the other hand, in my rats fasting for very long periods, the hypophysis (in the males), alimentary canal, testes and epididymi show a marked loss in weight, which does not occur in rats underfed for shorter periods.

The present experiments thus emphasize the fact that with advancing age, many of the different organs show a changing tendency in their growth reaction during underfeeding. The same organ (for example, the heart or alimentary canal) in young animals during the earlier periods of fasting may manifest a marked tendency toward continued growth while the increase in body weight is greatly retarded, but later may barely maintain itself, and finally perhaps even lose weight. Other organs, like the eyeballs, spinal cord and skeleton, continue to grow or maintain their weight with remarkable persistency; while still others (thymus) show a marked loss throughout all periods of inanition. These changes in weight in the various systems and organs of the young body during inanition are probably correlated with histological, chemical and physiological changes concerning which as yet but little is known.

SUMMARY

The more important results of the present investigation may be summarized briefly as follows:

Newborn albino rats are able to withstand separation from the mother for nearly one-half of the total time for the usual nursing period of three weeks, resulting in great retardation of the normal growth of the body as a whole.

During the underfeeding, the tail in the test rats elongates more rapidly than the body, thus producing relatively long-tailed individuals.

The eyelids in the test rats open at a body weight lower than in the controls, but the time of opening is somewhat delayed.

As to the body proportions, the weights of the extremities are

but slightly (if at all) changed in the test rats during both short and very long fasting periods. The head apparently increases slightly in weight, especially during the very long fasts, the increase being compensated by a slight decrease in the weight of the trunk.

The results for the various systems are as follows:

The skeleton shows a considerable increase in weight in the test rats. The growth apparently proceeds along the lines of normal development, as indicated by a decrease in water-content, the appearance of third molar teeth, and by formation and fusion of various epiphyses.

The musculature in the test rats increases slightly in weight in the majority of cases.

The visceral group (as a whole) shows a considerable increase in weight in the test rats underfed from birth to three, six and ten weeks of age. During the longer fasts there is apparently a less definite change, with a tendency to decrease in the majority of cases.

The integument remains practically normal in weight in the rats underfed from birth to three weeks, but shows a marked loss in weight later. Although the integument as a whole loses weight during underfeeding to ten weeks of age, nevertheless the external ear continues to grow considerably in size, and tends to assume the normal adult appearance. The continued growth of the ear is probably associated with the persistent growth tendency of its skeletal (cartilaginous) portion.

The 'remainder' appears to decrease in the rats underfed to three weeks of age, which is partly compensated for by skeletal increase. During the longer fasts there is a slight increase in the weight of the 'remainder' in the majority of cases.

In general, therefore, in young rats subjected to prolonged inanition, there appears to be a progressive tendency in the skeleton and (to a slight extent) in the musculature to increase in weight, counterbalanced by a decrease in the integument and viscera. The individual organs of the visceral group vary greatly, however.

The brain shows a very marked increase in weight in the test rats underfed to three weeks, and also to a slight extent at later periods.

The spinal cord shows a marked increase in weight in the test rats up to ten weeks of age, and also to a lesser degree in the longer periods.

There is a marked increase in the weight of the eyeballs in the test rats during both the short and the very long fasts.

The thyroid gland apparently remains practically unchanged in weight in the test rats at three, six, and ten weeks, but usually suffers a loss later.

The thymus shows a progressive decrease to about 80 per cent loss in weight in the test rats at ten weeks, with still greater loss (up to 94 per cent) at later periods.

There is little change in the weight of the heart in the test rats at three weeks. At six and ten weeks there is an apparent increase of 13 and 27 per cent respectively. During the longer fasting periods the heart apparently loses weight.

At three, six and ten weeks the lungs show a loss of about 29 per cent in the test rats. At the later periods there is an apparent increase which is of questionable significance.

The liver shows an apparent increase of 16, 70 and 64 per cent in weight in the test rats at three, six and ten weeks, respectively. During the very long fasts it is variable, with a tendency to decrease considerably in the majority of cases.

There is an apparent loss of 49 per cent in the weight of the spleen in the test rats at three weeks, while at six and ten weeks there is an apparent increase of about 27 per cent. During the later periods the spleen is variable, showing a tendency to decrease in most instances.

The alimentary canal, including contents, shows a loss in the test rats at three weeks of age, whereas at six and ten weeks there is an apparent increase. The changes are variable in the longer periods.

There is a marked increase in the weight of the empty alimentary canal in the test rats up to ten weeks. A reduction in weight, however, usually occurs during the very prolonged fasts.

The suprarenals in the test rats show a progressive increase in weight amounting to about 114 per cent at ten weeks, which usually is maintained, to a lesser degree, during the longer fasts, in the majority of cases. At ten weeks of age and also at subsequent periods the suprarenals are heavier in the test females than in the test males of corresponding age and body weight, thus indicating the appearance of sexual differentiation in weight during underfeeding.

There is an increase in the weight of the kidneys in the test rats at three, six, and ten weeks of age, but its variation from the normal appears doubtful at later periods.

The testes show a marked increase in weight in the test rats at three, six, and ten weeks, but later are variable, in some cases showing no change, and in other instances losing considerably in weight.

There is an increase in the weight of the epididymides of 95 and 14 per cent in the test rats at three and six weeks respectively. At ten weeks and later the epididymides apparently lose weight in the underfed rats.

The ovaries show a marked increase in the test rats at three, six and ten weeks of age, but are variable later, in most instances showing a tendency to increase.

There is an increase in the weight of the hypophysis in the test rats at three, six and ten weeks. Later the hypophysis is variable, showing a loss in the males and an increase in the females in most instances. In the very old test females the weight of the hypophysis is higher than in the corresponding males, indicating that sexual differentiation in the weight of the gland had occurred.

There is apparently no definite change in the weight of the pineal body during either short or very long periods of fasting.

In general, it therefore appears that in rats underfed from birth up to three, six and ten weeks, there is a marked increase in the weight of the spinal cord, eyeballs, liver, and stomach and intestines (empty). A less marked tendency to increase occurs in the brain (especially in the earliest period), heart (progressive increase), spleen (at six and ten weeks), intestinal contents (at six and ten weeks), suprarenals (progressive increase), kidneys, testes, epididymi (at three and six weeks, loss later), ovaries and hypophysis.

There is no marked change in the weights of the thyroid and pineal glands. A marked loss in weight occurs in the lungs and especially in the thymus. In the earliest period only (up to three weeks) there is a loss in the weight of the spleen and intestinal contents.

In the rats underfed for very long periods (starting at three weeks of age), the well-marked increase is maintained in the weight of the spinal cord, eyeballs, and usually of the suprarenals. The brain also shows a slight increase. In the case of the lungs, however, the apparent increase is of doubtful significance. There is apparently no marked change (or inconstant variability) in the weights of the intestinal contents, kidneys, ovaries, testes (?), and pineal body. The thyroid, thymus, heart, liver (variable), spleen (variable), alimentary canal (empty), epididymi, and hypophysis (in males) usually suffer a loss in weight during the long underfeeding periods.

LITERATURE CITED

ARON, HANS 1911 Nutrition and growth. Philippine Jour. Science, vol. 6, pp. 1–51.

1914 Untersuchungen über die Beeinflussung des Wachstums durch die Ernährung. Berliner klin. Woch., J. 51, S. 972–977.

VON BECHTEREW, W. 1895 Ueber den Einfluss des Hungerns auf die neugeborenen Thiere, insbesondere auf das Gewicht und die Entwickelung des Gehirns. Neurol. Centralblatt, Bd. 14, S. 810–817.

BIRK, W. 1911 Unterernährung und Längenwachstum beim neugeborenen Kinde. Berl. klin. Woch., Bd. 2, S. 1227.

BRÜNING, H. 1914 Experimentelle Studien über die Entwicklung neugeborener Tiere bei längerdauernder Trennung von der säugenden Mutter und nachheriger verschiedenartiger Ernährung. Jahrb. f. Kinderheilk. Bd. 80, S. 65–85.

DONALDSON, H. H. 1911 The effect of underfeeding on the percentage of water, on the ether-alcohol extract, and on the medullation in the central nervous system of the albino rat. Jour. Comp. Neur., vol. 21, no. 2.

1915 The Rat. Reference Tables and Data for the Albino Rat and the Norway Rat. Memoirs of The Wistar Institute of Anatomy and Biology, No. 6.

HARMS, W. 1909 Ueber den Einfluss des Hungers auf die Wirbelsäule der Tritonen. Verhandl. d. deutschen zool. Gesellschaft, 19. Versamml. S. 307–312.

HATAI, S. 1904 The effect of partial starvation on the brain of the white rat. Am. Jour. Physiol., vol. 12, no. 1.

1908 Preliminary note on the size and condition of the central nervous system in albino rats experimentally stunted. Jour. Comp. Neur., vol. 18, no. 2.

1913 On the weights of the abdominal and the thoracic viscera, the sex glands, ductless glands and the eyeballs of the albino rat (Mus norvegicus albinus) according to body-weight. Am. Jour. Anat., vol. 15, no. 1.

1914 On the weight of the thymus gland of the albino rat (Mus norvegicus albinus) according to age. Am. Jour. Anat., vol. 16, No. 2.

1915 The growth of the body and organs in albino rats fed with a lipoid-free ration. Anat. Rec., vol. 9, no. 1.

HESS, A. F. 1916 Infantile Scurvy III. Its influence on growth (length and weight). Amer. Jour. Diseases of Children, vol. 12, no. 2, p. 152.

HOSKINS, E. R. 1916 The growth of the body and organs of the albino rat as affected by feeding various ductless glands (thyroid, thymus, hypophysis and pineal). Jour. Exp. Zoöl., vol. 21, no. 3.

JACKSON, C. M. 1912 On the recognition of sex through external characters of the young rat. Biol. Bull., vol. 23.

1913 Postnatal growth and variability of the body and of the various organs in the albino rat. Am. Jour. Anat., vol. 15, no. 1.

JACKSON, C. M. 1915 a Effect of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. Amer. Jour. Anat., vol. 18, no. 1. Also abstracted in Proc. Amer. Assn. Anatomists. Anat. Rec., vol. 9, No. 1, p. 90.

1915 b Changes in the relative weights of the various parts, systems, and organs of young albino rats held at constant body-weight by underfeeding for various periods. Jour. Exp. Zoöl., vol. 19, no. 2. Also abstracted in Proc. Amer. Assn. Anatomists. Anat. Rec., vol. 9, no. 1, p. 91.

1917 Effects of inanition and refeeding upon the growth and structure of the hypophysis in the albino rat. Amer. Jour. Anat., vol. 21, no. 2.

JACKSON, C. M., AND LOWREY, L. G. 1912 On the relative growth of the component parts (head, trunk and extremities) and systems (skin, skeleton, musculature and viscera) of the albino rat. Anat. Rec., vol. 6, no. 12.

JOLLY, J., ET LEVIN S. 1911 a Sur les modifications de poids des organes lymphoïdes à la suite du jeune. C. R. S. Biol., T. 71.

1911 b Sur les modifications histologiques du thymus à la suite du jeune. C. R. S. Biol., T. 71.

JONSON, A. 1909 Studien uber die Thymusinvolution. Die akzidentelle Involution bei Hunger. Arch. f. mikr. Anat., Bd. 73, S. 390–443.

KING, H. D. 1915 Growth and variability in the body weight of the albino rat. Anat. Rec., vol. 9, no. 10.

LOWREY, L. G. 1913 The growth of the dry substance in the albino rat. Anat. Rec., vol. 7, no. 9.

MORGULIS, S. 1911 Studies of inanition in its bearing upon growth. I. Archiv f. Entw. d. Org., Bd. 32, H. 2.

STEWART, C. A. 1916 Growth of the body and of the various organs of young albino rats after inanition for various periods. Biol. Bull., vol. 31, no. 1, pp. 16–51. Also Abstracted in Anat. Rec., vol. 10, no. 3.

1917 Changes in the relative weights of the various parts, systems and organs of very young albino rats underfed for various periods. Abstract in Proc. Amer. Assn. of Anatomists, Anat. Rec., vol. 11, no. 6, pp. 410–411.

WATERS, H. J. 1908 a Capacity of animals to grow under adverse conditions. Proc. Soc. for the Promotion of Agricultural Science.

1908 b The influence of nutrition upon the animal form. Presented at the Thirtieth Meeting of the Society for the Promotion of Agricultural Science.

WATSON, C. 1910 Food and feeding in health and disease. Edinburgh and London. (In the Appendix, the results of several feeding experiments and investigations are republished from the Jour. of Physiol., etc.)

910.19 571

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, AUGUST 7

Reprinted from THE JOURNAL OF COMPARATIVE
NEUROLOGY, Vol. 29, No. 5, October, 1918

WEIGHTS OF VARIOUS PARTS OF THE BRAIN IN NORMAL AND UNDERFED ALBINO RATS AT DIFFERENT AGES

C. A. STEWART

Institute of Anatomy, University of Minnesota, Minneapolis

ONE FIGURE AND THREE TABLES

CONTENTS

Material and methods.......... 512
Brain.......... 515
Cerebrum.......... 519
Brain stem.......... 521
Cerebellum.......... 522
Olfactory bulbs.......... 523
Discussion.......... 524
Summary.......... 526
Bibliography.......... 527

In connection with an earlier study of the changes in the relative weights of the various organs in underfed albino rats (Stewart, '18 b), the brain was observed to manifest a remarkable tendency toward continued growth in very young animals, even when increase in body weight was prevented. The work has been extended in the present investigation to a more detailed consideration of the growth of separate parts of the brain in severely stunted rats. In addition, a considerable number of observations was made on the weights of different parts of the brain in normal rats at various ages. This phase of the work, however, was discontinued when I learned that Prof. H. H. Donaldson, of The Wistar Institute of Anatomy, Philadelphia, had undertaken the latter problem upon an extensive scale. Fortunately, we had each selected the same brain subdivisions for consideration, so that direct comparison of our results is possible. Dr. Donaldson has kindly furnished me with a record of his data for stock albino rats (presented in part in a Harvey Lecture, December, 1916) which in general are in agreement with my own observations upon normal animals. Only my own data are presented in this paper, however. This opportunity is taken to acknowledge my indebtedness to Prof. C. M. Jackson for valuable advice given during the course of the present experiments.

MATERIAL AND METHODS

In the present investigation the brains of 64 normal (31 males, 33 females, table 1), and of 29 test rats (19 males, 10 females, table 2) were used. Among the 64 normal rats are included 22 individuals which also served as the direct controls for the test rats. The rats used were all Albinos (Mus norvegicus albinus) from the colony in the Institute of Anatomy here.

The number and sex of the normal animals killed at the selected periods between birth and adult age are shown in table 1. Of the direct controls (table 2), 12 rats (8 ♂, 4 ♀) were killed at birth (average weight 4.7 to 5 grams), 9 rats (5 ♂, 4 ♀) at approximately 10 grams, and one male at 12 grams body weight.

The test rats were repeatedly starved by isolation from the mother for various periods as described elsewhere (Stewart, '18 b). In this manner 15 individuals (9 ♂, 6 ♀) were held approximately at birth weight for periods ranging from 5 to 18 days; and 13 rats (9 ♂, 4 ♀) were permitted to increase slightly in weight reaching approximately 10 grams at 3 weeks of age. In addition one male was weaned when 21 days old, body weight 10.5 grams, and was placed upon a limited diet of whole wheat (Graham) bread and whole milk. At 56 days of age this individual weighed only 12 grams.

It will be noted that the average final body weight is practically the same for the control and the test rats of each group (table 2). In comparing the data for the normal and the starved individuals, the slight differences existing in body weight have been disregarded. This seems justified since the error involved is small and cannot obscure the changes produced by the experimental conditions. Strictly speaking, however, a slight correction should be made as previously noted (Stewart, '18 b).

TABLE 1

Number and sex of rats, body weight and length, and weight of the brain with the percentage weights of its various subdivisions in normal rats at various ages

NUMBER, SEX, AND AGE OF RATS	GROSS BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	PER CENT OF BRAIN CEREBRUM	PER CENT OF BRAIN STEM	PER CENT OF BRAIN CEREBELLUM	PER CENT OF BRAIN OLFACTORY BULBS
	grams	*mm.*	*grams*				
8 ♂, 4 ♀, Newborn.....	4.86	48.6[1]	0.2086	64.4	29.3	3.65	2.51
1 ♀, 2 days............	5.40	50.0	0.2640	62.4	30.3	4.28	3.12
1 ♀, 4 days............	6.00	[5]	0.3545	67.6	25.6	5.64	1.31
1 ♀, 5 days............	8.50	58.0	0.4400	68.6	22.5	5.43	3.47
1 ♂, 6 days............	10.00	62.0	0.5135	69.4	22.4	5.36	2.75
1 ♂, 1 ♀, 8 days.......	9.80	62.0	0.6259	71.7	18.8	6.55	2.93
2 ♂, 1 ♀, 9 days........	10.5	66.5[2]	0.6969	71.4	19.1	6.32	3.15
1 ♂, 1 ♀, 10 days.......	10.35	65.0	0.6699	71.2	18.8	7.33	2.65
1 ♀, 11 days...........	9.50	62.0	0.7451	70.1	18.7	8.06	3.14
1 ♂, 12 days...........	11.00	[5]	0.6838	70.9	19.3	7.01	2.79
1 ♀, 14 days...........	15.00	[5]	0.9994	69.2	17.6	10.2	3.04
3 ♂, 3 ♀, 21.3 days.....	23.2	86.0[3]	1.2800	67.1	16.2	12.8	3.69
1 ♀, 28 days...........	28.0	97.0	1.2087	65.1	16.6	13.5	4.47
1 ♀, 35 days...........	33.0	105.0	1.3500	65.0	16.9	13.7	4.33
1 ♂, 2 ♀, 42.6 days.....	53.2	126.3	1.4389	64.3	17.3	13.2	4.83
1 ♂, 2 ♀, 49.6 days.....	41.5	115.6	1.2026	63.1	18.3	14.3	4.15
1 ♂, 63 days...........	73.5	141.0	1.5856	63.7	17.8	14.2	4.62
3 ♂, 4 ♀, 70 days......	96.1	149.0[4]	1.5962	63.6	18.0	13.9	4.58
1 ♂, 1 ♀, 114 days.....	142.5	176.0	1.6084	64.4	18.8	13.9	2.90
1 ♂, 1 ♀, 127 days.....	131.1	169.5	1.6839	61.4	19.4	14.1	5.01
2 ♂, 2 ♀, 143 days.....	164.7	182.5	1.7015	62.1	20.3	13.8	3.81
4 ♂, 4 ♀ { ♂ 357 days.. ♀ 454 days..	♂257.0 ♀213.0	♂212.0 ♀197.0	1.8100	60.8	21.8	14.6	2.92

[1] Average of 10 individuals.

[2] Average of 2 individuals.

[3] Average of 5 individuals.

[4] Average of 6 individuals.

[5] Not recorded.

The rats were killed either by chloroform or (in a few cases) by bleeding. The body (nose-anus) and tail lengths were immediately measured in the usual manner. The head was subsequently severed at a point immediately behind the foramen magnum. The brain was then carefully removed (care being exercised to preserve the cerebellar paraflocculi) and weighed in a closed container on balances accurate to 0.1 mgm.

The brain was next placed upon a moist plate of glass and its various parts dissected as follows. The olfactory bulbs were removed by a vertical incision at the point where they pass beneath the frontal lobes of the cerebrum. The cerebellum was then removed by severing its various crura or peduncles. Finally the cerebrum was separated from the brain stem by an incision immediately anterior to the superior colliculi passing through the anterior part of the crura cerebri. The cerebrum thus included the telencephalon (except olfactory lobes) and diencephalon, and the brain stem included the midbrain, pons, and medulla oblongata. The four brain subdivisions thus obtained were placed in a moist chamber and later carefully weighed in a closed container.

In all cases there is some difference between the total weight of the separate parts and the initial brain weight, due no doubt, either to evaporation from, or to fluid adhering to, the subdivisions weighed. Fortunately, however, the error is generally small and insignificant, especially as compared with the changes in weight that have occurred in the test rats. Based on the assumption that the error is probably distributed more or less proportionately among the various portions of the brain weighed, an attempt was made to correct the existing error n computing the percentage for each subdivision, by using the total weight of the separate parts rather than the original brain weight.

The observations in table 1 upon normal rats less than three weeks of age are grouped only in instances where there were two or more individuals of the same age. Later, when the brain growth is less rapid, the data have been averaged, grouping individuals differing a few (usually three or four) days in age. In the case of the eight adult rats only is there any considerable range in ages. Since the sexual difference in brain weight in animals of corresponding weight is comparatively small (Donaldson, '08, '09), the observations for males and females of each group have been combined. This is also justified by the relatively small number of observations, since the individual variations would obscure any existing difference according to sex.

In table 2 the data for the control groups and for the test rats are likewise grouped, only the averages being given. The original individual observations will be filed at The Wistar Institute of Anatomy and Biology (Philadelphia), where they may be consulted by those interested.

A preliminary report of the present investigation appeared in the Proceedings of the American Association of Anatomists, Minneapolis Meeting, December, 1917 (Stewart, '18a).

BRAIN

The weight of the entire brain (table 2) is considerably higher in the various groups of test rats than in the corresponding younger controls of the same body weight. In the fifteen individuals (9 ♂, 6 ♀) held at birth weight for various periods there is an increase from an average (sexes combined) of 0.2086 gram in the newborn controls to 0.4468 gram in the test rats, an increase of about 114 per cent. An inspection of the individual data in table 2 shows that the increase is greater in those rats held at maintenance for longer periods.

As may be observed in table 2, the body length also increases, although the body weight is held constant. The brain weight is also much greater in the stunted animals than in normal rats of the same body length, although the difference is not so great as when those of the same body weight are compared.

In the test rats weighing about 10 grams at 3 weeks the relative increase is less, amounting to approximately 33 per cent; while at 56 days with body weight at 12 grams the excess of brain weight in the test rats is about 30 per cent. In an earlier report (Stewart, '18 b) the brain in rats underfed from birth and weighing 10 grams at 3 weeks of age was found to exceed that in normal rats of corresponding body weight by about 60 per cent, which is considerably more than the excess obtained for a comparable group in the present series. In general, however, the results agree in showing a stronger growth tendency of the brain in the younger and smaller rats.

The increase in brain weight in spite of underfeeding with nearly stationary body weight is probably best shown in figure 1

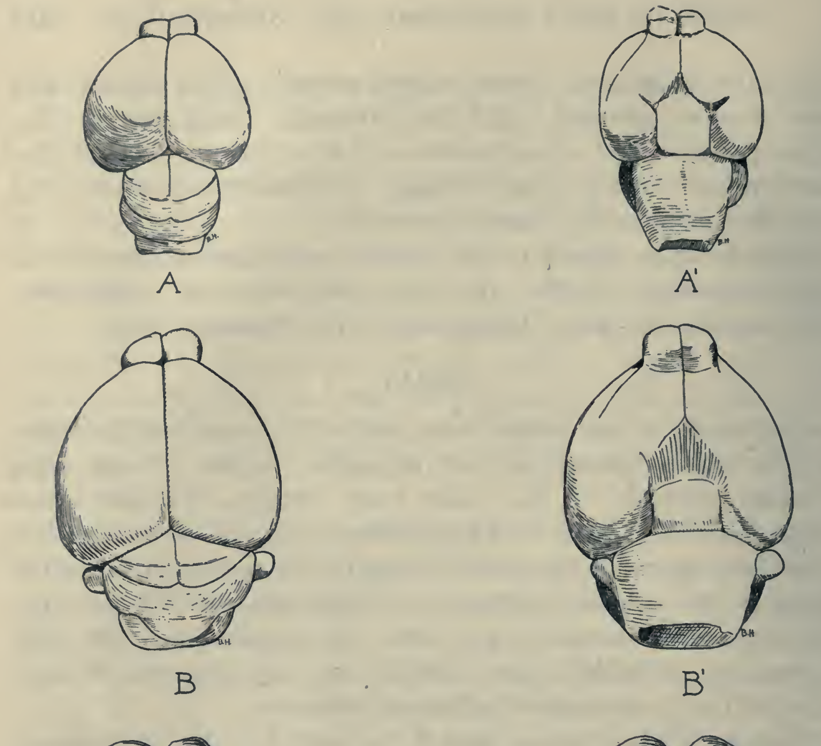


Fig. 1 *A* and *A'*, dorsal and ventral view, respectively, of brain of normal newborn rat. Body weight, 5.4 grams. Brain weight, 0.238 gram. × 3.

B and *B'*, dorsal and ventral view, respectively, of brain of rat kept at birth weight by underfeeding for 20 days. Body weight, 5 grams. Brain weight, 0.506 gram. × 3.

(*Continuation of explanation of figures on page opposite.*)

TABLE 2

Number, sex, weight, and length, with weights of the brain and its various parts in normal and underfed rats at different ages

NUMBER, SEX, AND AGE OF RATS	GROSS BODY WEIGHT	BODY LENGTH	BRAIN	CEREBRUM	STEM	CEREBELLUM	OLFACTORY BULBS
	grams	*mm.*	*grams*				
8 ♂, Control, newborn....	4.97	49.1[1]	0.2106	0.1375	0.0622	0.0079	0.0055
1 Test ♂, 5 days.........	4.7	56.0	0.3653	0.2481	0.1085	0.0213	0.0151
1 Test ♂, 6 days.........	5.5	56.0	0.3872	0.2724	0.0940	0.0232	0.0118
1 Test ♂, 7 days.........	4.6	55.0	0.3743	0.2563	0.1027	0.0213	0.0150
1 Test ♂, 12 days........	4.8	53.0	0.4711	0.3368	0.1014	0.0307	0.0154
1 Test ♂, 12 days........	5.8	57.0	0.5348	0.3742	0.1143	0.0356	0.0246
1 Test ♂, 12 days........	5.0	57.0	0.4790	0.3413	0.1134	0.0335	0.0192
1 Test ♂, 13 days........	5.5	59.0	0.5404	0.3845	0.1334	0.0346	0.0242
1 Test ♂, 14 days........	5.5	57.0	0.4920	0.3464	0.1062	0.0334	0.0220
1 Test ♂, 14 days........	5.8	60.0	0.4571	0.3247	0.1053	0.0291	0.0180
Average, 10.6 days......	5.24	56.7	0.4557	0.3205	0.1088	0.0292	0.0184
4 ♀, Newborn	4.65	47.5[2]	0.2046	0.1323	0.0611	0.0074	0.0048
1 Test ♀, 5 days.........	4.9	54.0	0.3603	0.2549	0.0999	0.0205	0.0149
1 Test ♀, 11 days........	4.0	52.0	0.3728	0.2564	0.1033	0.0212	0.0154
1 Test ♀, 12 days........	4.8	55.0	0.4434	0.3139	0.1018	0.0262	0.0180
1 Test ♀, 12 days........	5.0	54.0	0.5079	0.3444	0.1068	0.0328	0.0204
1 Test ♀, 13 days........	5.0	56.0	0.4019	0.2892	0.0872	0.0256	0.0142
1 Test ♀, 18 days........	5.1	54.0	0.5212	0.3732	0.1180	0.0415	0.0216
Average, 12 days.......	4.8	54.1	0.4346	0.3053	0.1028	0.0280	0.0174
5 Control ♂, 9 days......	10.2	63.0	0.6309	0.4589	0.1280	0.0396	0.0181
9 Test ♂, 22 days........	10.4	67.8	0.8837	0.6169	0.1578	0.0956	0.0265
4 Control ♀, 9.5 days.....	9.6	63.3	0.6845	0.4830	0.1282	0.0497	0.0212
4 Test ♀, 22 days........	9.6	65.9	0.8444	0.5894	0.1477	0.0896	0.0229
1 Control ♂, 9 days......	12.0	69.0	0.7324	0.5304	0.1340	0.0556	0.0240
1 Test ♂, 56 days........	12.0	77.0	0.9544	0.6312	0.1856	0.1210	0.0410

[1] Average of 7 individuals.

[2] Average of 3 individuals.

On comparison with the brain of the newborn rat (*A* and *A′*), it is evident that during underfeeding (maintenance) not only the brain as a whole, but also the olfactory bulbs and tracts, cerebral hemispheres, colliculi, cerebellum and flocculi, tuber cinereum, pons, and medulla continue to grow in young rats in spite of practically no change in body weight for 20 days. Comparison with the normal brain at 20 days (*C* and *C″*), however, shows that the growth of the various parts of the brain mentioned above, while considerable, nevertheless has been greatly retarded.

C and *C′*, dorsal and ventral view, respectively, of brain of normal rat at 20 days of age. Body weight, 17.5 grams. Brain weight, 1.047 grams. × 3.

(*a*, *a′*, newborn control; *b*, *b′*, test rat). As compared with the control of the same body weight, not only the various subdivisions weighed, but also the olfactory tracts, tuber cinereum, corpora quadrigemina, and especially the para flocculi are evidently much larger in the starved rat.

Although considerable brain growth thus occurs during maintenance of constant body weight in young rats, nevertheless it occurs at a greatly retarded rate in comparison with the normal growth during the corresponding period of time. This is so well shown in figure 1 (*b*, *b′* and *c*, *c′*) that a lengthy discussion is unnecessary.

TABLE 3

Comparison of relative intensity of growth of various parts of the brain in normal and test rats

	PERCENTAGE BY WHICH THE WEIGHT OF THE VARIOUS PARTS OF THE BRAIN			
	At 11 days exceeds that at birth		At 3 weeks exceeds that in normal 10-gram rats	
	Normal	Test	Normal	Test
Cerebellum	696	272	274	113
Olfactory bulbs	351	240	144	30
Cerebrum	293	131	85	29
Brain stem	130	72	63	20

In older rats held at maintenance for various periods, investigators in general have noted practically no change in the brain weight. Thus Hatai ('08) found the brain weight in stunted rats to be practically identical with that for normal younger rats of the same body weight. In a large series of rats studied by Donaldson ('11), after maintenance from 30 to 51 days of age, the brain exceeded the calculated initial weight by only 3.6 per cent. Jackson ('15 a) and Stewart ('16) conclude that there is practically no change in the weight of the brain in rats held at maintenance for various periods starting at 3 weeks of age. Holt ('17) noted a slight increase in brain weight in undersized rats fed upon an unsuitable diet of whole corn after the period of weaning.

Aron ('11) publishes data showing in a few instances the brain weight in greatly stunted dogs practically equal to that in normal heavier dogs of the same age. However, no observations were made concerning the initial brain weight at the beginning of the experiment and the extent of the brain growth which apparently occurred during the underfeeding is therefore uncertain.

It is interesting to note that Variot and Lassabliere ('09) found the growth of the brain in underfed infants to be retarded less than the growth in body weight, the brain thus increasing at the expense of other tissues of the body. This observation agrees with my own results for young albino rats.

During severe starvation a slight decrease in the weight of the brain was noted by Bechterew ('95) in puppies and kittens, and by Hatai ('04) in young rats. Acute and chronic inanition in adult rats causes but little if any loss in absolute brain weight (Jackson, '15 b). A very complete summary of the literature bearing upon the effect of inanition upon the brain is given by Jackson ('15 b).

CEREBRUM

If the percentages are calculated from the combined weight of the separate brain parts, it appears that the cerebrum (table 1) (telencephalon and diencephalon, excluding olfactory bulbs) during normal growth increases from an average of slightly more than 64 per cent of the entire brain at birth (sexes combined) to a relative maximum of approximately 71 per cent during the early part of the second week. Thereafter, although increasing in absolute weight, the cerebrum forms a progressively smaller proportion of the brain, decreasing to an average of approximately 67 per cent (sexes combined) at three weeks, and to about 61 per cent at one year and later. My results agree fairly well with those of Hatai ('15) for adult individuals of approximately similar body length, with those of Sugita ('17) for the rat during the first 150 days, and also with the unpublished data of Donaldson (personal communication). Slight differences appear which are presumably due partly to experimental error and partly to normal variability in the size of the brain segments.

In the stunted rats kept at birth weight for various periods, and also in those weighing approximately 10 and 12 grams at three and eight weeks, respectively, the weight of the cerebrum (table 2) considerably exceeds that of the normal younger controls of corresponding body weight. For the first group (sexes combined) there is an increase from an average of 0.1358 gram in the controls to 0.3144 gram in the test rats, an increase of more than 131 per cent. In the test rats at three weeks (22 days) the increase in the cerebrum is relatively less, amounting to approximately 29 per cent, and at eight weeks it has decreased to about 19 per cent.

As to relative proportions, the percentage weight of the cerebrum is slightly higher in the test rats kept at birth weight various periods than in the controls, the apparent increase being from an average of approximately 64 per cent of the combined weight of the separate parts in the latter to 67 per cent in the stunted individuals. The range in the test rats is from about 63 per cent to 69 per cent, increasing in general with the length of the experiment. If we now compare the corresponding change in relative proportion of the cerebrum during normal growth, it is evident that with the increase in brain weight from birth there is normally an increase in the percentage that the cerebrum forms of the entire brain, similar and practically equal to that noted in the brain of equal size in the stunted rats.

In the test rats weighing about 10 grams at three weeks, however, there is apparently a slight decrease (71 to 69 per cent) in the relative size of the cerebrum as compared with the controls of the same body weight. Likewise during normal growth there is a similar decrease in the percentage weight of the cerebrum while the brain weight is increasing from about 0.6500 gram to 0.8700 gram. At eight weeks of age the cerebrum in the test rats, though absolutely larger, is relatively smaller than in the control. This change likewise is probably associated with the usual tendency toward declining relative size of the cerebrum in normal brains of corresponding weight, although the difference in this case is greater than would be expected from the apparent change during normal growth.

In general, therefore, the data indicate that during the persistent growth of the brain, in very young rats stunted by underfeeding, the cerebrum (telencephalon and diencephalon) maintains the same relative size as in the normal brain of corresponding weight.

BRAIN STEM

The percentage weights (calculated from the combined weights of separate brain subdivisions) for the brain stem (including midbrain, pons, and medulla oblongata) show a relative decrease from an average of about 29 per cent of the normal brain at birth to about 16 per cent at three weeks. Subsequently the growth of the brain stem is more rapid than that of the brain as a whole, resulting in a gradual increase in relative weight to an average of about 22 per cent in the adult rats. The normal initial decrease and subsequent increase in the relative size of the brain stem is in agreement with the observations by Sugita ('17) and Donaldson (unpublished data). As compared with adult animals of corresponding body length, my results correspond fairly well with those obtained by Hatai ('15).

In the stunted rats held at birth weight for various periods, and also in the other groups of test animals, the weight of the brain stem (table 2) in all cases considerably exceeds that in the younger controls of the same body weight. In the first group there is an increase from an average of 0.0618 gram (sexes combined) for the controls to 0.1064 gram in the test rats, an increase of approximately 72 per cent. In the test rats at three and eight weeks of age, the increase in brain stem weight amounts to about 20 and 39 per cent, respectively.

As to relative proportions, the brain stem in the rats held at birth weight apparently decreases from about 29 per cent (in the controls) to about 22 per cent of the brain weight. In the test rats at 22 days the brain stem weight has further decreased to about 18 per cent, as compared with 19 per cent in the controls of similar body weight. In the test rat at 56 days, however, the brain stem has slightly increased to 19 per cent (18 per cent in the control).

In these cases, however, the relative size of the brain stem in the brain of the stunted rats corresponds approximately to that in normal brains of the same weight. The only exception is that of the single test rat in which the brain stem should be relatively slightly smaller than in the control, according to the change found in the normal brains of corresponding weights. The difference is slight, however, and may be obscured in this case by individual variation or experimental error.

On the whole, therefore, it appears that during the persistent growth of the brain, in very young rats stunted by underfeeding, the brain stem (midbrain, pons, and medulla) maintains approximately the same relative size as in the normal brain of corresponding weight.

CEREBELLUM

Calculations from the data obtained show that during postnatal growth the cerebellum (table 1) apparently increases rapidly from an average of about 3.7 per cent of the total weight of the separate parts of the brain at birth (sexes combined) to about 14 per cent at seven weeks of age, and maintains approximately this relative weight in the adult albino rat. In general these results agree fairly well with those obtained by Hatai ('15) (for adult rats of body length similar to that of my adult controls), by Sugita ('17) (from birth to 150 days of age), and by Donaldson (unpublished data).

In the rats underfed for various periods the cerebellum (table 2) shows a remarkable growth. In the individuals held at birth weight the cerebellum has increased from an average of 0.0077 gram in the controls (sexes combined), to 0.0287 gram, an increase of over 272 per cent. At three and eight weeks of age the increase in the test rats is approximately 113 and 118 per cent, respectively, as compared with the younger controls of the same body weight.

As to relative proportions, the cerebellum is in all cases found relatively much larger in the brain of the test rats, in comparison with that in control rats of the same body weight. If the percentage of the brain weight formed by the cerebellum in the test rats (table 2) is compared with that in normal brains (table 1) of the same weight, however, the agreement is surprisingly close. It is therefore apparent that during the persistent growth of the brain in underfed young rats the cerebellum, like the segments previously considered, maintains approximately the same relative size as in the normal brain of corresponding weight.

OLFACTORY BULBS

Calculations from my data (table 1) indicate that the olfactory bulbs, although rather variable, in general increase from an approximate average (sexes combined) of 2.5 per cent of the total weight of the separate parts of the brain at birth, to about 3.7 per cent at three weeks, and probably reach a relative maximum of about 4.8 per cent at six or seven weeks of age. Subsequently their relative weight decreases in the majority of cases, reaching about 2.9 per cent in the adult. Attention should be called to the fact that the data indicate not only a relative decrease, but even an absolute loss in the weight of the olfactory bulbs in the older rats. In general my results agree with those obtained by Hatai ('15), Sugita ('17), Holt ('17), and Donaldson (unpublished data) for normal albino rats, the existing differences probably being due partly to normal variability and partly to experimental error.

In the stunted rats the olfactory bulbs greatly exceed those in the younger controls of the same weight (table 2). For the group held at birth weight there is an increase of nearly 240 per cent. For the other test animals at three and eight weeks of age the increase is less marked, amounting to 30 and 71 per cent, respectively.

The percentage that the olfactory bulbs form of the entire brain weight, especially in the case of the individuals kept at birth weight for various periods, averages higher than that for the newborn controls. According to my data, accompanying an increase in brain weight from approximately 0.2100 gram to 0.4500 gram there is normally a considerable increase in the relative weight of the olfactory bulbs, although apparently not so great as in the stunted rats with brains of corresponding weight.

Thus the olfactory bulbs appear relatively larger in the stunted rats than in normal rats of the same brain weight.

For the group fasting three weeks the relative increase is slight and inconstant. In the normal rats with corresponding brain weight (0.6309 to 0.8837 gram) the relative weight of the olfactory bulbs is likewise nearly stationary, though somewhat variable. This is in agreement with Holt ('17), who found the relative proportions of the olfactory bulbs to remain practically unchanged in rats undersized after four and eight weeks of feeding upon an unsuitable diet of whole corn.

The data for my rat underfed from birth to eight weeks indicate an apparent increase in the relative weight of the olfactory bulbs, which is in accordance with the general tendency toward an increase in the relative size of olfactory bulbs in normal rats with brains of corresponding weight. Miss Holt noted a tendency for the bulbs to increase in relative weight during prolonged defective feeding in rats weighing about 50 grams.

On the whole it therefore appears that during the persistent growth of the brain in underfed young rats the olfactory bulbs tend to maintain a relative size similar to that in the normal brain of corresponding weight. In the youngest and smallest group, however, they apparently become relatively hypertrophied and appear relatively larger than in normal animals with the same brain weight.

DISCUSSION

Quite uniformly the results of experiments have shown that the brain demonstrates a marked ability to grow when increase in body weight is prevented by underfeeding only in very young animals and at a time when the normal growth of the brain is very pronounced. There is, therefore, apparently a definite relation between the increase in size accomplished during starvation and the normal growth power possessed by the organ at the time when underfeeding is commenced That this dependency upon the intensity of the growth impulse applies also to the various parts of the brain is evident upon comparison of the relative rapidity of growth of the various parts of the brain in young rats.

As is shown in table 3, in both the normal and test rats, the cerebellum manifests the strongest growth power, the olfactory bulbs, cerebrum, and brain stem following in the order mentioned. The perfect agreement in this respect between the control and test individuals can hardly be considered a mere coincidence, but more probably is the expression of inherent normal tendencies.

Furthermore, it has been pointed out that with the growth of the brain in the stunted rats, the various portions of the brain undergo practically the same changes in relative size as found in normal animals of the same brain weight. The olfactory bulbs which show an overgrowth (in the younger group) apparently form the only notable exception to this rule. In general, therefore, it appears that normal growth tendencies foreshadow the character and the amount of the changes that accompany growth of the brain during underfeeding. In other words, the growth of the brain in the stunted rats appears normal, so far as the changes in the size of the constituent parts is concerned.

The tendency for the different brain subdivisions to maintain largely the normal proportions during starvation is in marked contrast to the change in the relative weights produced among various organs of the body as to result of underfeeding. Jackson ('15, p. 152) also noted that in some cases (e.g., liver, alimentary canal) there is a certain degree of parallelism between the normal growth tendency and the behavior of organ weight in young rats when the body weight is held constant. Furthermore, the greatest number of organs showing growth during maintenance (constant body weight) occurs in very young rats during the normal period of most rapid growth (Stewart, '18). The above-mentioned principle therefore applies not only to the brain and its various subdivisions, but also to many other organs. However, there are certain exceptions to this rule, as pointed out by Jackson.

It is interesting to note that the marked growth of the brain in rats stunted by underfeeding occurs only at a period when the normal increase in size is still due partly to cell multiplication, especially in the cerebellum (Allen, '12). This is a phase of the inanition problem worthy of further investigation.

SUMMARY

1. The weights of various parts of the brain were studied in 64 normal rats at various ages, and also in 29 test animals, of which 15 individuals were held at birth weight by underfeeding for periods ranging from 5 to 18 days of age, 13 rats were permitted to increase slightly in weight reaching approximately 10 grams at 3 weeks, and one male weighed 12 grams at 56 days of age.

2. According to the data available, the cerebrum (excluding olfactory bulbs) increases from a normal average of slightly more than 64 per cent of the entire brain at birth, to a maximum of about 71 per cent during the early part of the second week, but subsequently forms a progressively smaller proportion of the brain, decreasing to an average of about 61 per cent in the adult.

The brain stem (including midbrain, pons, and medulla oblongata) decreases from a normal average slightly exceeding 29 per cent of the brain at birth to about 16 per cent at 3 weeks, but later increases reaching a relative weight of about 22 per cent in adult animals.

The cerebellum increases rapidly from an apparent average of 3.7 per cent of the entire brain at birth to about 14 per cent at 7 weeks of age, and thereafter.

The olfactory bulbs, while variable, in general increase from an average of about 2.5 per cent at birth to 3.7 per cent at 3 weeks, and probably reach a relative maximum of slightly more than 4.5 per cent at 6 or 7 weeks of age. Subsequently there is a gradual decrease in relative weight to about 2.9 per cent in the adult rat. The data indicate not only a relative decrease, but even an absolute loss in the weight of the olfactory bulbs in the older rats.

3. For the stunted rats the weight of the brain as a whole in the individuals held at birth weight for various periods averaged 114 per cent higher than that in the controls, whereas the excess in test rats weighing 10 grams at 3 weeks and 12 grams at 8 weeks of age was 33 and 30 per cent, respectively.

As shown in figure 1, the increase is shared not only by the various brain parts dissected and weighed, but also by the olfactory tracts, tuber cinereum, colliculi, and the paraflocculi.

Of the various parts of the brain, the weights of the cerebellum, olfactory bulbs, cerebrum, and brain stem exceed those for the control rats of corresponding weight in the order mentioned, the greatest change occurring in the individuals kept at birth weight for various periods. In this group of individuals the different parts of the brain in the order above listed show an increase of 272, 240, 131, and 72 per cent, respectively.

In the persistent growth of the brain in the young rats stunted by underfeeding, the various parts of the brain in general preserve approximately the same relative weight as in normal individuals of the same brain weight. The olfactory bulbs apparently form an exception to this rule, as they become abnormally large in the younger group of stunted rats. This apparent hypertrophy may be due, however, to experimental error.

BIBLIOGRAPHY

Allen, E. 1912 The cessation of mitosis in the central nervous system of the albino rat. Jour. Comp. Neur., vol. 22, no. 6, pp. 547–568.

Aron, Hans 1911 Nutrition and growth. Philippine Jour. Science, vol. 6, no. 1, pp. 1–51.

Donaldson, H. H. 1908 A comparison of the albino rat with man in respect to the growth of the brain and of the spinal cord. Jour. Comp. Neur., vol. 18, no. 4, pp. 345–392.

1909 On the relation of the body length to the body weight and to the weight of the brain and of the spinal cord in the albino rat (Mus norvegicus var. albus). Jour. Comp. Neur., vol. 19, no. 2, pp. 155–167.

Hatai, S. 1908 Preliminary note on the size and condition of the central nervous system in albino rats experimentally stunted. Jour. Comp. Neur., vol. 18, no. 2, pp. 151–155.

1915 The growth of the body and organs in albino rats fed with a lipoid-free ration. Anat. Rec., vol. 9, no. 1, pp. 1–20.

Holt, C. M. 1917 Studies on the olfactory bulbs of the albino rat. In two parts: I. Effect of a defective diet and of exercise. II. Number of cells in bulb. Jour. Comp. Neur., vol. 27, no. 2, pp. 201–259.

Jackson, C. M. 1915 Changes in the relative weights of various parts, systems, and organs of young albino rats held at constant body weight by underfeeding for various periods. Jour. Exp. Zool., vol. 19, No. 2, pp. 99–156.

1915 e Effect of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. Am. Jour. Anat., vol. 18, no. 1. Also abstracted in Proc. Amer. Assn. Anatomists. Anat. Rec., vol. 9, no. 1, p. 90.

STEWART, C. A. 1916 Growth of the body and of the various organs of young albino rats after inanition for various periods. Biol. Bull., vol. 31, no. 1, pp. 16–51.

1918 a Weights of the various parts of the brain in normal and underfed albino rats at different ages. (Abstract.) Proc. Amer. Assn. Anatomists. Anat. Rec., vol. 14, no. 1, p. 51.

1918 b Changes in the relative weights of the various parts, systems, and organs of young albino rats underfed for various periods. Jour. Exp. Zool., vol. 25, no. 2, pp. 301–353.

SUGITA, N. 1917 Comparative studies on the growth of the cerebral cortex. I. On the changes in the size and shape of the cerebrum during postnatal growth of the brain. Albino rat. Jour. Comp. Neur., vol. 28, no. 3, pp. 495–510.

VARIOT ET LASSABLIÈRE, P. 1909 Autonomie du developpement de l'encéphale, dans les retards de la croissance chex les jeunes enfants. C. R. Soc. Biol., Paris, T. 66, pp. 106–108.

Ho. 20. 67/

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. 48, No. 1,
February, 1919

CHANGES IN THE WEIGHTS OF THE VARIOUS PARTS, SYSTEMS AND ORGANS IN ALBINO RATS KEPT AT BIRTH WEIGHT BY UNDERFEEDING FOR VARIOUS PERIODS

CHESTER A. STEWART

From the Institute of Anatomy, University of Minnesota, Minneapolis

Received for publication December 6, 1918

It has been shown that in young animals stunted by underfeeding certain of the systems and organs of the body show a remarkable tendency to continue their growth, others remain nearly unchanged, while still others lose weight to a variable extent (Aron, '11 and '14; Jackson, '15b; Stewart, '16 and '18a). The object of the present paper is to show the changes which occur in the weights of different organs and systems of the body in albino rats kept at birth weight (about 5 grams) by underfeeding for various periods, and also to compare these changes with those previously observed in larger rats during various periods and degrees of stunting.

Material and methods. In the present experiment 23 albino rats (Mus norvegicus albinus) were used, including 8 newborn controls (5 males, 3 females) and 15 test rats (8 males, 7 females). The sex and body weight for each individual rat is shown in table 1. The letters "St" indicate the series; the number preceding the decimal point indicates the litter to which the rat belongs and the number following the decimal point designates the individual rat.

The test rats were stunted from birth for periods ranging from 11 to 22 days of age (average 16 days) by subjecting them to repeated periods of fasting. This was accomplished by removing the test animals from the mother at successive periods as described in a previous paper (Stewart, '18a). Under normal conditions the body weight of the albino rat at 16 days (the average age of the test rats at the end of the experiment) would have at least trebled the birth weight. Thus the maintenance at birth weight for this period represents a condition of very severe inanition, which is maintained only with great difficulty. Many animals died during the course of the experiment and were not utilized on account of the possibility of post mortem changes. The general plan adopted was to adjust the length of the alternate fasting and nursing periods in such manner as to keep the starved individuals practically at birth weight. In this respect the experiments were very successful for, as is evident from tables 1 and 2, the average weights of the control and test animals are nearly equal.

TABLE 1

Individual data for the albino rats used in the experiments

RAT NO.	USE AND SEX	BIRTH WEIGHT	WEIGHT AT AUTOPSY	AGE
		grams	*grams*	*days*
St 237.2	Control M	4.9	4.9	0
St 246.6	Control M	5.0	5.0	0
St 240.5	Control M	5.1	5.1	0
St 239.5	Control M	5.4	5.4	0
St 242.4	Control M	5.9	5.9	0
Average		5.3	5.3	
St 236.3	Test M	5.0	4.8	12
St 203.6	Test M	4.1	5.0	14
St 240.3	Test M	5.0	5.0	22
St 240.11	Test M	4.9	5.0	21
St 240.7	Test M	4.8	5.0	18
St 236.1	Test M	5.3	5.5	14
St 214.4	Test M	5.5	5.7	14
St 235.8	Test M	5.8	5.8	12
Average		5.0	5.2	16
St 238.1	Control F	5.0	5.0	0
St 240.1	Control F	5.1	5.1	0
St 239.7	Control F	5.5	5.5	0
Average		5.2	5.2	
St 215.5	Test F	5.0	4.0	11
St 235.1	Test F	4.8	4.8	12
St 235.4	Test F	5.0	5.0	12
St 239.10	Test F	5.4	5.0	20
St 214.6	Test F	5.5	5.0	13
St 237.3	Test F	4.1	5.1	18
St 214.2	Test F	4.6	5.1	18
Average		4.9	4.9	15

TABLE 2

Showing the changes in average body length, tail ratio and weights of the various systems and organs in albino rats held at constant body weight by underfeeding from birth to 11 to 22 days of age

	MALES		FEMALES		SEXES COMBINED		AVERAGE PER CENT OF CHANGE
	Controls 5 newborn	8 Underfed 11–22 days	Controls 3 newborn	7 Underfed 11–22 days	Average of controls	Average of test rats	
Gross body weight, grams	5.26	5.22	5.20	4.86	5.24	5.05	−4
Net body weight, grams	5.08	5.03	4.98	4.64	5.04	4.85	−4
Body length, mm	50.4	57.0	50.3	54.7	50.4	55.9	+11
Tail ratio, per cent	31.3	40.5	32.4	43.6	31.7	41.9	+46
Head, grams	1.04	1.55	1.03	1.46	1.04	1.51	+45
Visceral group, grams	0.876	1.340	0.866	1.205	0.873	1.277	+46
Integument, grams	0.758	1.001	0.750	0.881	0.755	0.945	+25
Musculature and skeleton, grams	1.91	2.11	1.86	1.88	1.89	2.00	+6
"Remainder," grams	1.53	0.586	1.51	0.680	1.52	0.630	−59
Testes, grams	0.0027	0.0128			0.0027	0.0128	+374
Epididymis, grams	0.0016	0.0052			0.0016	0.0052	+225
Eyeballs, grams	0.024	0.057	0.023	0.060	0.024	0.059	+146
Brain, grams	0.217	0.525	0.231	0.474	0.223	0.501	+125
Kidneys, grams	0.0416	0.0874	0.0483	0.0794	0.0441	0.0837	+90
Spinal cord, grams	0.035	0.067	0.034	0.061	0.035	0.064	+83
Stomach and intestines (empty) grams	0.151	0.221	0.162	0.212	0.155	0.217	+40
Spleen, grams	0.012	0.019	0.011	0.012	0.012	0.016	+33
Hypophysis, grams	0.00056	0.00071	0.00047	0.00063	0.00052	0.00067	+29
Heart, grams	0.031	0.041	0.031	0.036	0.031	0.039	+26
Pineal body, grams	0.00028	0.00037	0.0003	0.00033	0.00029	0.00035	+21
Stomach and intestines (full) grams	0.334	0.413	0.383	0.427	0.352	0.420	+19
Thyroid, grams	0.00126	0.0015	0.0014	0.0013	0.0013	0.0014	+8
Ovaries, grams			0.00110	0.00116	0.00110	0.00116	+5
Lung, grams	0.088	0.097	0.083	0.079	0.086	0.089	+3
Suprarenals, grams	0.0021	0.0021	0.0017	0.0019	0.0019	0.0020	+5
Liver, grams	0.260	0.199	0.229	0.182	0.248	0.191	−23
Thymus, grams	0.0079	0.0042	0.0079	0.0038	0.0079	0.0040	−49

In comparing the data from the starved individuals with those from the controls, the slight difference in body weight (average about 4 per cent less in the test animals) has generally been disregarded, for the resultant error is small and does not modify the main conclusions based upon the data obtained.

The experiments were made in the Institute of Anatomy, University of Minnesota. The warm room in which the stunted rats were kept while fasting, and also the autopsy technique used are described in a previous paper (Stewart, '18a). The net "body weight" is the gross body weight less the weight of the contents of the alimentary canal.

For economy of space only average data are included in tables 2 and 3. However, copies of the original individual observations will be deposited at the Wistar Institute of Anatomy and Biology, Philadelphia, where they may be consulted if so desired.

Body length and tail length. Immediately after death the body (nose-anus) length and tail length of each rat were measured and recorded. Averages of the individual ratios $\frac{\text{(tail length)}}{\text{(body length)}}$ were computed from the data thus obtained and expressed as percentages (table 2). The ratio of tail length to body length is known as the "tail ratio."

According to the data in table 2, the body length of the rats kept at birth weight for 11 to 22 days (57.0 mm.) averages 11 per cent greater than the body length of the newborn controls (50.4 mm.). This indicates persistent skeletal growth in the young starved rats even though no increase in body weight was permitted. This result agrees with the previous observations by Jackson ('15b) and Stewart ('18a). Continued increase in body length during stunting by underfeeding has been observed not only in the rat, but also in calves (Waters, '08), and in dogs (Aron, '11). Also in malnourished children a strong persistent tendency to growth in body length (skeleton) has been noted by Variot ('07), Birk ('11), Aron ('14), Hess ('16) and others, even in cases where the body weight is stationary. This question is discussed more fully by Jackson and Stewart ('18).

The results (table 2) show the tail ratio to be considerably higher in the test than in the control rats. There is an apaprent increase in the tail length from a normal average of approximately 0.32 (31.7 per cent) of the body length in the newborn controls (sexes combined) to about 0.42 (41.9 per cent) in the starved individuals. This corresponds to an average increase of about 46 per cent in the absolute length of the tail, as compared with an increase of 11 per cent in the body length. It is therefore evident that while the body continues to increase in length even during maintenance at birth weight, the elongation of the tail under these circumstances continues still more rapidly than that of the body, thus producing relatively long-tailed individuals.

Furthermore the data (table 2) show the tail to be relatively longer in the female than in the male rat, not only in the control but also in the test animals. Jackson ('15b) and Stewart ('18a) likewise noted the tail to become relatively long in young rats held at maintenance, and with a persistent tendency to average slightly longer in the female than in the male.

The relative elongation of the tail during underfeeding, although retarded in rate, apparently is an expression of the normal growth process, for according to Jackson ('15b) the tail ratio of the albino rat normally increases from an average of 0.36 (36 per cent of body length) at birth to 0.66 at 3 weeks, and is slightly greater in the female.

Head and body parts. The weight of the head (table 2) averages considerably higher in the test rats of each sex than in the younger controls of corresponding body weight. The combined data indicate an increase from an average of about 1.04 gram in the controls to approximately 1.51 gram in the test rats, an increase of about 45 per cent. As will be evident later, the increase (0.47 gram) can be attributed only partly to an increase in the weight of the brain and eyeballs. The extent to which the additional excess is shared by the integument, skeleton and musculature is uncertain from the data available, for no direct observations were made upon these parts for the head separately.

In older and larger rats underfed for various periods Jackson ('15b) and Stewart ('18a) noted a tendency for the head to increase in the majority of instances, even when the body weight was held constant. In general, however, the changes were not so marked as those observed in the present series of young individuals, in whom the intensity of growth is relatively much stronger. In a group of rats stunted by underfeeding from birth to 3 weeks, (body weight doubled), the average increase in the weight of the head was 16 per cent, as compared with normal controls of the same body weight (Stewart, '18a). This evidence of a relatively stronger growth tendency in the head region in the younger animals recalls the general law of cranio-caudal progression in growth and development, as formulated by Jackson ('09).

No direct observations were made with regard to the weight of the trunk and extremities, but with an increase in the weight of the head during maintenance of constant body weight, the trunk and extremities collectively have necessarily decreased to a corresponding extent.

Visceral group, integument, musculature and skeleton, and "remainder." The weights of the visceral group (including abdominal and thoracic viscera, spinal cord, brain and eyeballs) and of the integument average considerably higher in the test than in the control rats of corresponding body weight, the average increase (sexes combined) amounting to 46 per cent for the viscera and 25 per cent for the integument. Also the musculature and skeleton together show a slight excess weight in the stunted animals in this case, however, amounting to only 6 per cent. The extent to which the change is shared by the skeleton and the musculature individually is uncertain for in this series the two were not weighed separately.

The "remainder" is obtained by deducting the weight of the integument, viscera, skeleton and musculature from the net body weight. It thus includes salivary and lymph glands, tongue, oesophagus, fat and body fluid but not the intestinal contents. A marked loss in weight of the "remainder" has occurred during underfeeding. The data indicate a decrease from an average of 1.52 gram in the controls to 0.63 gram in the test rats of corresponding body weight, a loss of about 59 per cent. The loss suffered by the "remainder" practically compensates for the combined increase observed for the above mentioned systems (correction being made for difference in average body weight).

A review of the literature dealing with the effect of inanition in general upon the parts and systems as well as the individual organs of the body in the rat and other animals is given by Jackson ('15a, '15b) and Stewart ('16, '18a).[1]

Organs gaining weight during fasting. From the data in table 2, it is evident that a considerable number of organs show a remarkable tendency toward continued growth in weight in young rats, even when an increase in body weight is prevented. The organs are arranged in order of their growth intensity when the body is held at birth weight in the first column of table 3. The most striking growth is noted in the case of the testes, epididymis, eyeballs and brain, the increase in the weight of these organs amounting to 374, 225, 146 and 125 per cent respectively. Of the other organs, the kidneys, spinal cord, empty stomach and intestines, spleen, hypophysis, heart and pineal body also increase in weight considerably during maintenance of constant body weight from birth, the average percentages of increase being 90, 83, 40, 33, 29, 26 and 21 per cent respectively. The integument with

---

[1] Statements in regard to the "remainder" after long periods of fasting (Stewart, '18a) should be changed to agree with the data, the latter being correct as published. Thus on page 328, line 8, "higher" should be changed to "lower." Also on page 331, line 6, change "six" and "ten" to "three" and "six" (weeks of age, etc.), respectively.

TABLE 3

Changes in weight of organs in underfed albino rats compared with normal at same body weight

	MAINTENANCE FROM BIRTH TO 11-22 DAYS BODY WT. 5 GRAMS. (STEWART)	STUNTING FROM BIRTH TO 3 WKS. BODY WT. 10 GRAMS. (STEWART '18a)	STUNTING FROM BIRTH TO 10 WKS. BODY WT. 15 GRAMS. (STEWART '18a)	MAINTENANCE FROM 3 WKS. TO 10 WKS. BODY WT. 24 GRAMS. (JACKSON '15b)	STUNTING FROM 3 WKS. TO 400 DAYS BODY WT. 50 GRAMS. (STEWART '18a)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Testes	+374	+188	+51	+34	−42
Epididymis	+225	+95	−6	−4	−32
Eyeballs	+146	+41	+66	+50	+73
Brain	+125	+60	+8	−0.5	+4
Kidneys	+90	+21	+38	+4	−6
Spinal cord	+83	+70	+70	+36	+40
Visc. group	+46	+28	+38	+0.9	+1
Head	+45	+16	+4	+6	+26
Stomach and intestines	+40	+17	+100	+28	−27
Spleen	+33	−49	+24	−42	−5
Hypophysis	+29	+33	+24	M +18 F +19	F +10 M −15
Heart	+26	−5	+27	−0.6	−10
Integument	+25	+0	−48	−36	−11
Pineal body	+21	−4	+12		−8
Thyroid gland	+8	+5	+4	−24	−36
Skeleton	+6	+19	+24	+28	+32
Musculature		+8	+10	+3	+4
Ovaries	+5	+83	+54	−27	+17
Suprarenals	+5	+60	+114	M +12 F +39	M +36 F +66
Lungs	+3	−26	−26	−15	+28 (?)
Liver	−23	+17	+64	+10	−7
Thymus	−49	−30	−80	−90	−90
"Remainder"	−59	−40	−23	−1.9	−33

Note. The data in the first column are from table 1.

an increase of 25 per cent and the stomach and intestines including contents with an increase of 19 per cent also belong to this group.

The empty stomach and intestines show an increase from 0.155 gram in the controls to 0.217 gram in the test rats, a gain of about 40 per cent. When the contents are included with the stomach and intestines there is an apparent increase in the starved rats from a normal average of 0.352 gram to 0.420 gram, the increase amounting to about 19 per cent. The smaller gain recorded for the full than for the empty stomach and intestines is evidently due to the fact that the weight of the intestinal contents remained practically unchanged during the underfeeding, increasing from a normal average of 0.197 gram to 0.203 gram in the test rats, a gain of only about 3 per cent.

In the case of the pineal gland the apparent increase of 21 per cent in the test rats may be somewhat questionable, especially when the extremely small size of the organ is taken into consideration. Very small errors in technique of removal and weighing the organ may be responsible for the apparent increase in its weight.

On the whole, however, it is evident that during the severe inanition required to hold the body at birth weight the above mentioned organs continue to increase in weight at the expense of other parts of the body. In some instances the increase accomplished during starvation is very remarkable.

Organs remaining nearly constant in weight during fasting. In a smaller group of organs (see table 2) there is practically no growth in the rats kept at birth weight. The percentage increases (table 3, first column) are as follows: thyroid gland, 8; ovaries, 5; suprarenals, 5; and lungs, 3. The skeleton and musculature, which together show an increase of 6 per cent, also belong in this group. The average weights for these organs differ from those for the control animals by less than 10 per cent although in each case the average small difference indicates a slight apparent increase in weight of the corresponding organ.

Organs losing weight during fasting. Of the organs weighed individually, only two, the liver and thymus (tables 2 and 3) were observed to lose in weight in the rats kept at birth weight. The average data (sexes combined) indicate a loss of 23 per cent for the liver and 49 per cent for the thymus. As previously noted, the greatest loss is found in the "remainder" (decrease of 59 per cent) which, together with that of the liver and thymus, must counterbalance the gain in the other parts of the body since the body weight is held approximately constant.

DISCUSSION AND CONCLUSIONS

On comparing the results of the present experiments with those previously obtained in similar experiments upon older and larger animals a more complete survey than was hitherto available is obtained of the weight changes which occur among the various organs and parts of the body at various ages during inanition. This comparison brings out very strikingly the changing reaction undergone by the various individual organs at different ages when the individual is stunted in growth by underfeeding. The extent and character of this change for individual organs and systems is so well shown in table 3 that a detailed discussion of the individual organs is unnecessary. Special attention is called to certain points, however.

The visceral group as a whole averages 46 per cent increase in weight in the rats held at birth weight, as shown in column 1 of table 3. At later ages, and with longer periods of underfeeding (as shown in the successive columns of table 3) the tendency of the visceral group to increase persists, but becomes weaker. In the rats underfed beginning at the age of 3 weeks (last two columns of table 2), the visceral group barely holds its own in weight. In comparison with this general trend in the visceral group, the individual organs show great variability.

The testes show a remarkable increase of 374 per cent in rats kept at birth weight for various periods. In later periods of underfeeding the tendency to increase in the weight of the testes is progressively weaker, while in rats weighing approximately 50 grams at 400 days a loss of 42 per cent is recorded. Other organs, like the epididymis, brain, kidneys and alimentary canal, during the earlier periods of underfeeding exhibit a marked growth capacity, but later (like the visceral group as a whole) are barely able to maintain themselves, or in some cases actually suffer a loss in weight, under the conditions of the experiment. The eyeballs and spinal cord, however, not only increase markedly in weight in the younger underfed groups, but also tend to maintain strong growth tendencies with considerable persistency, even in the older groups. The thymus at all periods shows a marked loss in weight during underfeeding, while the other organs are variable.

The above mentioned increases in the weights of various organs and parts of the body during underfeeding have been observed to be accompanied also by continued structural development of apparently normal character. Thus in the case of the skeleton (Jackson, '15b and Stewart, '18a) continued growth during stunting has been shown to be accompanied by continued development of apparently normal character (increasing proportion of solids, formation and fusion of epiphyses, development of third molar teeth). A progressive differentiation of the integument and external ear and opening of the eyelids before reaching the body weight at which this normally occurs has also been noted in young rats stunted by underfeeding (Stewart, '18a). The growth of the brain under similar conditions is likewise accompanied by normal differentiation of the parts as shown by the relative weight of the various segments (Stewart, '18b).

While the growth tendencies of the skeleton and musculature are apparently weaker in the present series held at birth weight than in subsequent periods, the integument and the visceral group show decidedly stronger growth capacities than at later periods of underfeeding (see table 3). A relatively enormous growth capacity is exhibited by certain organs during this earliest period. In older and larger rats considerable growth has previously been observed in the case of certain organs during underfeeding, but such remarkable changes as found in the present series (rats kept at birth weight) with increase of 374, 225 and 146 per cent (testes, epididymis and eyeballs respectively) have not hitherto been observed. As a general rule it appears that in most cases the growth tendency of organs during underfeeding is strongest during the youngest stages.

When individual organs are considered, however, it will be noted that there are some exceptions to this rule. For example, the liver shows a loss of 23 per cent in rats kept at birth weight (11 to 22 days) whereas in underfed animals weighing 10 and 15 grams at 3 and 10 weeks of age there is an increase of 17 and 64 per cent respectively. Also in the case of the ovaries and suprarenals there is apparently a lower growth tendency noted in the youngest group as compared with the marked increase found at 3 and 10 weeks of age in rats weighing 10 and 15 grams, respectively. Other organs, such as the spleen and lungs, are somewhat irregular.

In comparing the results of the present experiment with earlier investigations on the effects of underfeeding at later periods, the following generalizations seem justified. The "remainder" suffers a very heavy loss in rats held at birth weight by underfeeding, while the skeleton and musculature show a very feeble gain as compared with the tendency at somewhat later periods. The tendency of continued growth in the integument and visceral group during inanition is stronger in newborn rats than in older and larger animals subjected to similar conditions. This question is discussed in greater detail in the paper by Jackson and Stewart ('18).

The number of individual viscera showing an increase in weight during underfeeding is greater in the young rats than at later periods. While most of the individual organs (like the group as a whole) show a decreasing intensity of growth at later periods of underfeeding, there are several exceptions to the rule, as previously noted.

SUMMARY

1. The weights of the various organs, systems and parts of the body were studied in 8 newborn controls and 15 test rats kept at birth weight by underfeeding for periods ending at 11 to 22 days of age.

2. The underfed newborn rats become relatively long-tailed individuals, as previously found in similar experiments on older animals.

3. During maintenance at birth weight the head increases about 45 per cent in weight, compensated for by a corresponding decrease in the trunk and extremities. This is also similar to the results at later stages.

4. The visceral group increases 46 per cent, the integument 25 per cent and the musculature and skeleton (together) 6 per cent in the test rats, counterbalanced by a corresponding decrease of 59 per cent in the "remainder." The integument and visceral group thus show much stronger growth tendencies than in similar underfeeding experiments at later ages. The musculature and skeleton have apparently weaker growth tendencies than later, while the loss in the "remainder" is relatively greater than at later periods.

5. The individual organs in the test rats, as compared with those in newborn rats of corresponding weight show the following percentage increases in weight: testes, 374; epididymis, 225; eyeballs, 146; brain, 125; kidneys, 90; spinal cord, 83; stomach and intestines (empty) 40; spleen, 33; hypophysis, 29; heart, 26; pineal gland, 21; stomach and intestines (with contents), 19.

In the rats kept at birth weight the thyroid gland, ovaries, lungs and suprarenals remain practically unchanged in weight (gain of 8, 5, 5 and 3 per cent respectively); whereas the liver and thymus suffer a loss of 23 and 49 per cent respectively.

6. These changes in rats held at birth weight by underfeeding resemble in general those previously found in somewhat larger and older stunted animals (Jackson, '15b; Stewart, '18a; Jackson and Stewart, '18). Certain differences appear, however, as shown in table 3.

BIBLIOGRAPHY

Aron, H. '11. Philippine Journ. Sci., vi, 1.
'14. Berl. klin. Wochenschr., li, 972.
Birk, W. '11. Berl. klin. Wochenschr., Bd. ii, 1227.
Hess, A. F. '16. Amer. Journ. Diseases Children, xii, 152.
Jackson, C. M. '09. Amer. Journ. Anat., ix.
'15a. Amer. Journ. Anat., xviii. Also abstracted in Anat. Rec., ix, 90.
'15b. Journ. Exper. Zoöl., xix. Also abstracted in Anat. Rec., ix, 91.
Jackson, C. M. and C. A. Stewart. '18. Minnesota Medicine, i.
Stewart, C. A. '16. Biol. Bull., xxxi, 16. Also abstracted in Anat. Rec., x.
'18a. Journ. Exper. Zoöl., xxv. Also abstracted in Anat. Rec., xi, 410.
'18b. Journ. Exper. Neurol.
Variot, G. '07. Bull. et Mém. Soc. méd. d. hôpit. de Paris, xxiv, 1347. Also in Bull. soc. de pediatr. de Paris, ix, 363.
Waters, H. J. '08a. Proc. Soc. for the Promotion of Agric. Sci.
'08b. Presented at the 30th Meeting Soc. for the Promotion of Agric. Sci.

University of Minnesota.
Dept. of Anatomy.
Contributions.

P
Med
M

157477
Minnesota, University of. Dept. of Anatomy
Contributions.
Vol.6(1918/19)

Biological
& Medical
Serials

University of Toronto
Library

Biological
& Medical
Serials

DO NOT
REMOVE
THE
CARD
FROM
THIS
POCKET